APPENDIX 3

Probst et al., (1992) DNA Cell Biol.

DNA AND CELL BIOLOGY
Volume 11, Number 1, 1992
Mary Ann Liebert, Inc., Publishers
Pp. 1-20

REVIEW ARTICLE

Sequence Alignment of the G-Protein Coupled Receptor Superfamily

WILLIAM C. PROBST,*,‡ LENORE A. SNYDER,*,§ DAVID I. SCHUSTER,‡ JÜRGEN BROSIUS,* and STUART C. SEALFON*,†

ABSTRACT

The multitude of G-protein coupled receptor (GPR) superfamily cDNAs recently isolated has exceeded the number of receptor subtypes anticipated by pharmacological studies. Analysis of the sequence similarities and unique features of the members of this family is valuable for designing strategies to isolate related cDNAs, for developing hypotheses concerning substrate–ligand and receptor–effector interactions, and for undertanding the evolution of these genes. We have compiled and aligned the 74 unique amino acid sequences published to date and review the present understanding of the structural motifs contributing to ligand binding and G-protein coupling.

INTRODUCTION

THE CLONING of a great number of receptors and channels has revealed that many of these critical membrane proteins can be grouped into gene superfamilies based on sequence and structural similarities. One of these superfamilies comprises the G-protein coupled receptors (GPRs). Although the signal transduction mechanism is not known for all members of the gene family, in most cases receptor stimulation induces activation of a guanine nucleotide binding protein or G-protein. In 1982 the complete protein sequence of the visual pigment bovine rhodopsin was determined (Ovchinnikov *et al.*, 1982). Its predicted structure, containing an extracellular amino terminus and seven hydrophobic membrane spanning α-helices (Hargrave *et al.*, 1983), was remarkably similar to that previously identified by electron diffraction and sequence analysis for bacteriorhodopsin (Unwin and Henderson, 1975; Engelman *et al.*, 1980). The subsequent molecular cloning of four human opsins (Nathans and Hogness, 1984; Nathans *et al.*, 1986) and the hamster β-adrenergic receptor (Dixon *et al.*, 1986) again revealed these structural features that have become the hallmark of this gene family (Fig. 1).

The number of GPRs that have been cloned is increasing rapidly; at present 74 distinct GPR sequences have been published. GPR cloning has led to the stable high-level expression of these receptor subtypes in mammalian cell lines, a preparation that has greatly aided the pharmacological characterization of these receptors. Molecular biological alteration of receptor sequences and expression in cell lines has provided much of our knowledge concerning the functional role of particular receptor regions and residues.

We have aligned all the available amino acid sequences of the members of this family (Fig. 2). This compilation should prove useful for designing cloning strategies for other GPRs. Indeed, many GPRs, among them the dopamine receptors (Bunzow *et al.*, 1988; Dearry *et al.*, 1990), the adenosine receptors (Libert *et al.*, 1989b), and the cannabinoid receptor (Matsuda *et al.*, 1990), have been cloned *via* approaches relying on sequence similarity. In addition, this sequence alignment may facilitate the formulation of hypotheses concerning the role of certain protein sequences in determining ligand binding, regulation, and G-protein specificity of the receptors. Comparison of the structure of the genes for these receptors can provide insight into the evolution of this gene family.

*Fishberg Research Center for Neurobiology, †Department of Neurology, Mount Sinai School of Medicine, New York, NY 10029.
‡New York University, Department of Chemistry, New York, NY 10003.
§The Graduate School and University Center of the City University of New York, Ph.D. Program in Biology, New York, NY 10036-8099.

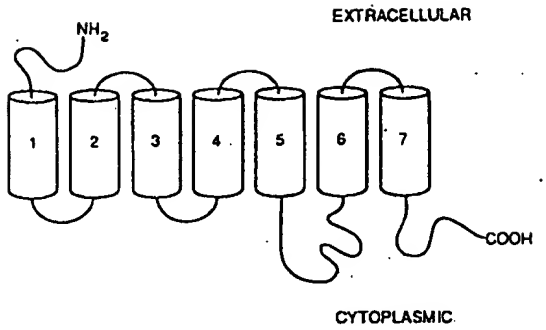


FIG. 1. The topography of G-protein linked receptors. Cylinders represent transmembrane α-helices. Extracellular and cytoplasmic sides of the plasma membrane are indicated.

The sequences were aligned manually, relying on invariate residues and published computer-generated sequence alignments. Several of the sequences, such as the FC5R receptor, have not yet been proven to represent GPRs. They are included in the alignment, however, because their sequences identify them as members of this superfamily. If sequences for the same receptor subtype of more than one species have been published, we have included only the sequence of the highest species. The sequences are organized into subgroups based on ligand type, *i.e.*, muscarinic receptors, catecholamine receptors, *etc.*

GENERAL STRUCTURAL FEATURES

All of the proteins are single polypeptide chains. The shortest sequence represents the rat *mas* oncogene (324 amino acids) and the longest sequence represents the human thyroid-stimulating hormone receptor (744 amino acids). The predicted protein structures contain seven stretches of 20–30 hydrophobic amino acids which are believed to form membrane-spanning α-helices. These helices are referred to as transmembrane domains 1–7 (TM 1–TM 7). This predicted structure, based on hydropathy analysis, has been supported by electron diffraction analysis for bacteriorhodopsin (Henderson *et al.*, 1990) and proteolytic cleavage studies for rhodopsin and the β_1-adrenergic receptor (Hargrave *et al.*, 1982; Dohlman *et al.*, 1988). The proteins have extracellular amino termini and cytoplasmic carboxyl termini.

The areas of greatest homology among the GPRs are in the seven transmembrane regions. Some residues are found in virtually all GPRs and may mediate the tertiary structure required for functional activity (Hulme *et al.*, 1990; Hibert *et al.*, 1991). Particularly well conserved are several proline residues in TM 4, 5, 6, and 7. These residues most likely introduce kinks in the α-helices and may be important in the formation of the binding pocket (Applebury and Hargrave, 1986; Findlay and Eliopoulos, 1990; Dahl *et al.*, 1991; Hibert *et al.*, 1991). Other well-conserved residues include a glycine, an asparagine, and a valine in TM 1; a leucine, two alanines, and an aspartate in TM 2; an isoleucine in TM3; a tryptophan in TM 4; a phenylalanine and a tryptophan in TM 6; and an asparagine and a tyrosine in TM 7 (see Fig. 3). Certain conserved residues are replaced in particular subfamilies. For example, the TM 6 conserved tryptophan is replaced by methionine in the glycoprotein hormone receptors (Fig. 2).

Most GPRs have single conserved cysteine residues in each of the first two extracellular loops that are believed to form a disulfide bond that stabilizes the functional protein structure (see Fig. 3). Mutation of either of these conserved cysteine residues markedly alters the function of rhodopsin, muscarinic, and β-adrenergic receptors (Dixon *et al.*, 1987a; Karnik *et al.*, 1988; Fraser, 1989; Hulme *et al.*, 1990). The most highly conserved intracellular sequence is the aspartate-arginine-tyrosine triplet adjacent to TM 3 which has been implicated in signal transduction (see below). The arginine of this triplet is invariant, and the aspartate and tyrosine are conservatively replaced in several GPRs.

The amino termini of these proteins vary greatly in length. They range from as few as seven residues in the adenosine A_3 receptor to over 300 residues for the glycoprotein hormone receptors. Overall, there is little sequence homology among the receptors in the first extracellular domain. The amino termini of nearly all the GPRs contain consensus sequences (N-X-S/T) for *N*-glycosylation (Kornfeld and Kornfeld, 1985). Rhodopsin, the α_2-adrenergic, the β_1-adrenergic, and the β_2-adrenergic receptors are all glycosylated at several of these sites (Hargrave, 1977; Strasser *et al.*, 1984; Benovic *et al.*, 1987b; Dohlman *et al.*, 1987; Regan, 1988). Glycosylation may contribute to the proper expression of membrane proteins (for review, see Kornfeld and Kornfeld, 1985). Deletion of the glycosylated domains of the β_2-adrenergic receptor decreased the level of receptor expression but did not alter ligand binding (Dixon *et al.*, 1987b). Inhibition of glycosylation diminished muscarinic receptor expression (Liles and Nathanson, 1986). The thyroid-stimulating hormone receptor contains six potential glycosylation sites. Mutational analysis demonstrated that two of these sites are required for the expression of functional receptor (Russo *et al.*, 1991). Some receptors with short amino termini (the A_1 and A_2 adenosine and the α_{2B}-adrenergic receptors) do not contain amino-terminal asparagine glycosylation sites.

Phosphorylation and palmitoylation of carboxy-terminal sites can influence the signal transduction of some GPRs. Most GPRs contain potential phosphorylation sites in the third cytoplasmic loop and/or carboxyl terminus. For several receptors, phosphorylation by protein kinase A and specific receptor kinases mediates receptor desensitization (see Intracellular Coupling below, for discussion). Two adjacent cysteine residues in the carboxyl terminus of rhodopsin and one in the β_2-adrenergic receptor are palmitoylated (Ovchinnikov *et al.*, 1988; O'Dowd *et al.*, 1989a). The hydrophobicity profile of the GPRs predict seven TM domains and thus three intracellular loops (Fig. 1). The palmitate on carboxy-terminal cysteine(s) would be expected to insert into the membrane, thereby forming an additional cytosolic loop which may influence receptor mobility (Findlay and Eliopoulos, 1990) or G-protein coupling (O'Dowd *et al.*, 1989a).

FIG. 2. Amino acid sequence alignment of the GPR superfamily. The putative transmembrane domains are enclosed in boxes. The precise boundaries of the TM domains are not known with certainty. Dashes have been introduced for the purpose of alignment. Amino acids omitted from nonconserved regions are indicated by numbers in parentheses.

1. Dictyostelium cAMP receptor (Klein *et al*,. 1988)
2. Dog adenosine A2 receptor (RDC8) (Libert *et al*., 1989b)
3. Dog adenosine A1 receptor (RDC7) (Libert *et al*., 1989b)

4. Human m1 muscarinic acetylcholine receptor (Peralta *et al*., 1987)
5. Human m2 muscarinic acetylcholine receptor (Peralta *et al*., 1987)
6. Human m3 muscarinic acetylcholine receptor (Peralta *et al*., 1987)
7. Human m4 muscarinic acetylcholine receptor (Peralta *et al*., 1987)
8. Human m5 muscarinic acetylcholine receptor (Bonner *et al*., 1988)

9. Human beta 1 adrenergic receptor (Frielle *et al*., 1987)
10. Human beta 2 adrenergic receptor (Kobilka *et al*., 1987a)
11. Human beta 3 adrenergic receptor (Emorine *et al*., 1989)
12. Cow alpha 1 adrenergic receptor (Schwinn *et al*., 1990)
13. Rat alpha 1B adrenergic receptor (Voigt, *et al*., 1990)
14. Human alpha 2 C4 adrenergic receptor (Regan *et al*., 1988)
15. Human alpha 2 C2 adrenergic receptor (Lomasney *et al*., 1990)
16. Human alpha 2 C10 adrenergic receptor (Kobilka *et al*., 1987c)
17. Rat alpha 2 adrenergic receptor R2O (Lanier *et al*., 1991)
18. Drosophila octopamine receptor (Arakawa *et al*., 1990)
19. Human dopamine D1 receptor (Dearry *et al*., 1990)
20. Human dopamine D5 receptor (Sunahara *et al*., 1991)
21. Human dopamine D2 receptor (Grandy *et al*., 1989)
22. Human dopamine D3 receptor (Giros *et al*., 1990)
23. Human dopamine D4 receptor (Van Tol *et al*., 1991)
24. Human serotonin 1d receptor [RDC4] (Hamblin and Metcalf, 1991)
25. Human serotonin 1a receptor (Kobilka *et al*., 1987b)
26. Rat serotonin 1c receptor (Julius *et al*., 1988)
27. Rat serotonin 2 receptor (Julius *et al*., 1990)
28. Human histamine H2 receptor (Gantz *et al*., 1991)

29. Human N-formyl peptide receptor (Boulay *et al*., 1990)
30. Human C5a anaphylatoxin receptor. (Gerard and Gerard, 1991)
31. Human thrombin receptor (Vu *et al*., 1991)
32. Human thromboxane A2 receptor (Hirata *et al*., 1991)
33. Human IL-8 receptor (Murphy and Tiffany, 1991)
34. Guinea-pig platelet-activating factor receptor (Honda *et al*, 1991)
35. Cow endothelin 1 receptor (Arai *et al*., 1990)
36. Rat non-isopeptide selective endothelin receptor (Sakurai *et al*., 1990)
37. Mouse bombesin/gastrin releasing peptide receptor (Spindel *et al*., 1991)
38. Rat neuromedin B preferring bombesin receptor (Wada *et al*., 1991)
39. Human vasoactive intestinal peptide (Sreedharan *et al*., 1991)
40. Rat neurotensin receptor (Tanaka *et al*., 1990)
41. Rat bradykinin receptor (McEachern *et al*., 1991)
42. Mouse thyrotropin-releasing hormone receptor (Straub *et al*., 1990)
43. Human neurokinin A (SK) receptor (Gerard *et al*., 1990)
44. Rat substance P receptor (Yokota *et al*., 1989)
45. Rat neuromedin K receptor (Shigemoto *et al*., 1990)
46. Bovine adrenal angiotensin II type-1 receptor (Sasaki *et al*. 1991)
47. Human mas oncogene (angiotensin) receptor (Young *et al*., 1986)

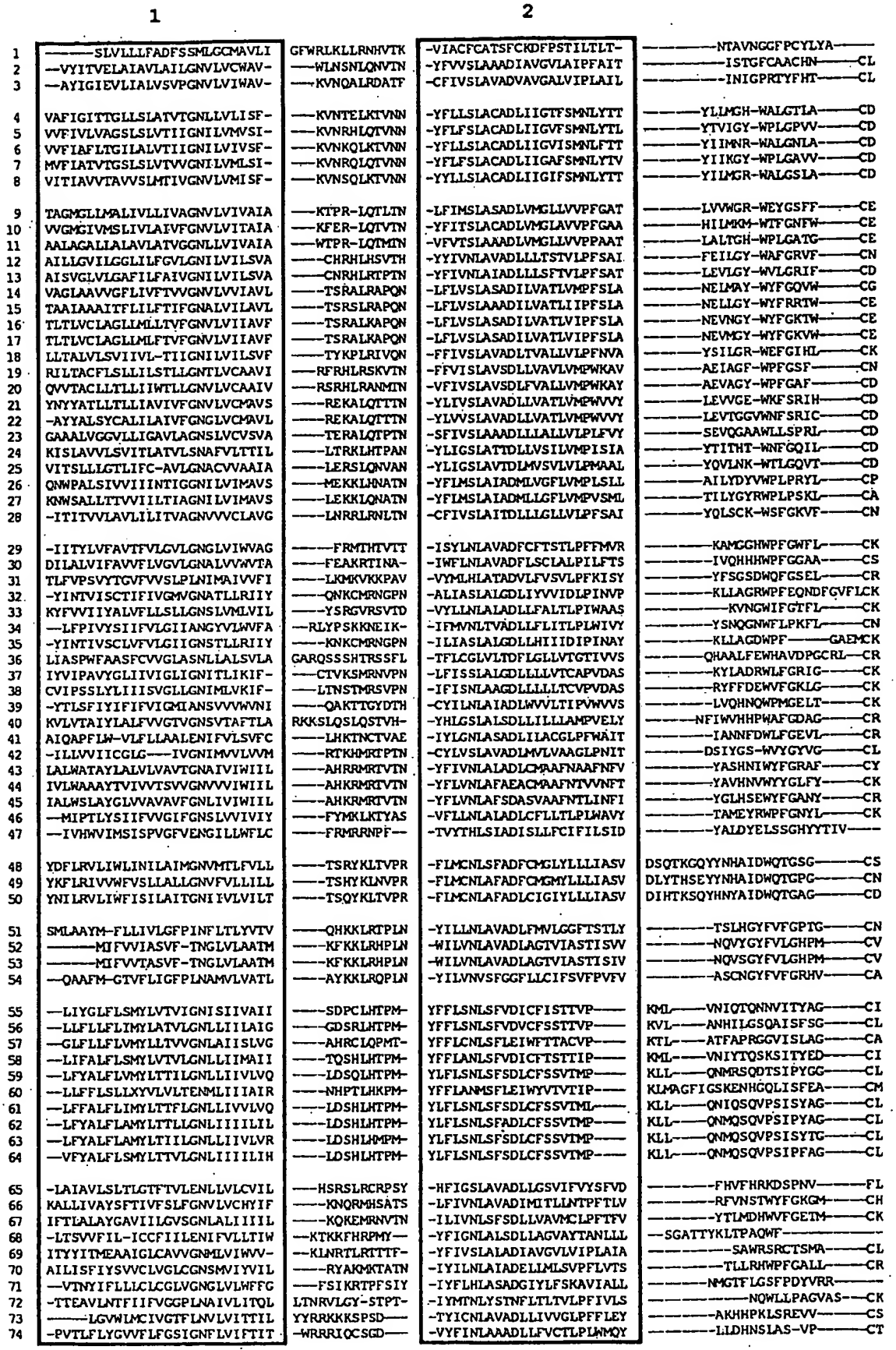
48. Human lutropin-choriogonadotropin receptor (Frazier *et al*., 1990)
49. Human thyrotropin receptor (Libert *et al*., 1989a)
50. Human follicle stimulating hormone receptor (Minegish *et al*., 1991)

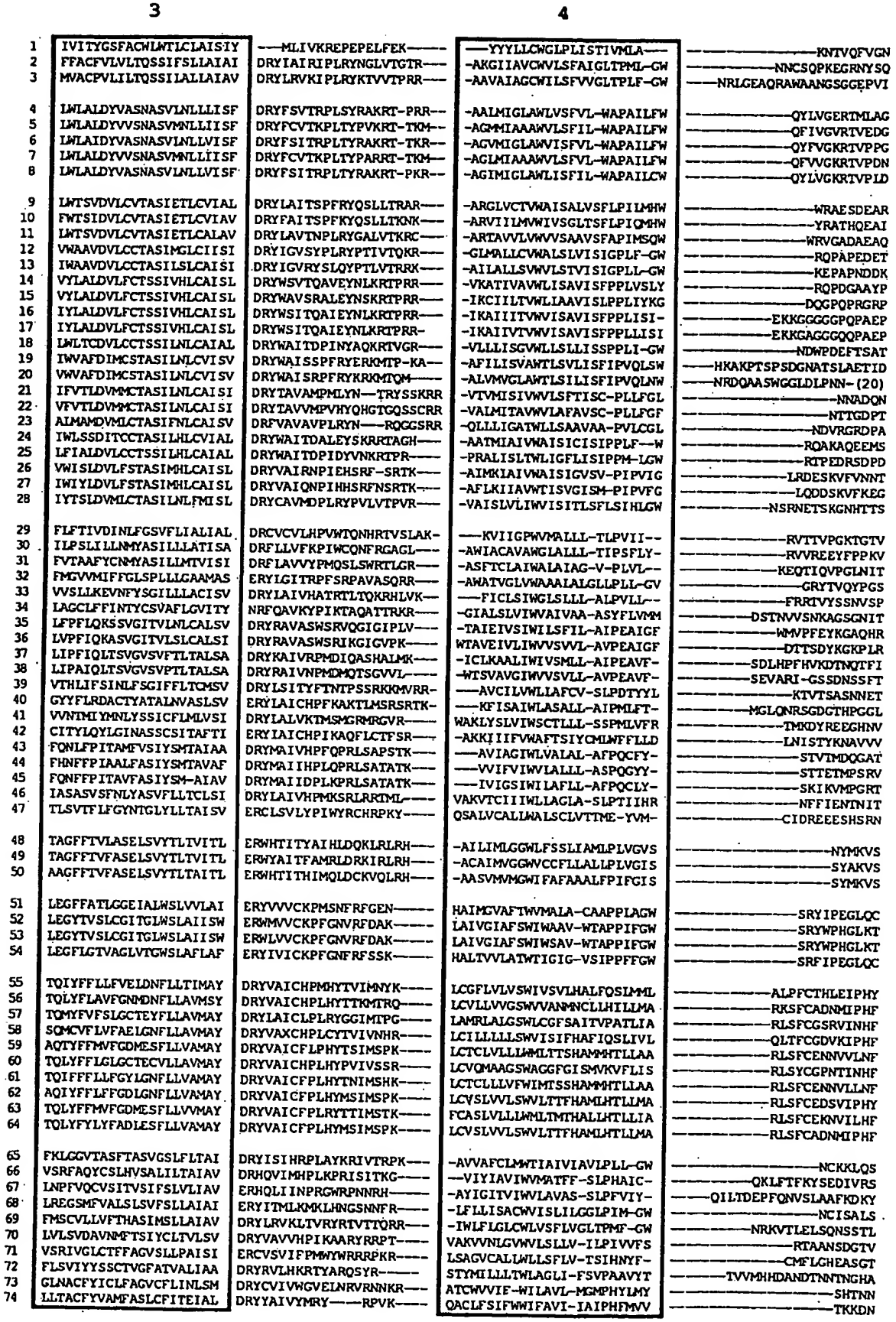
51. Human rhodopsin (Nathans and Hogness, 1984)
52. Human green opsin (Nathans *et al*., 1986)
53. Human red opsin (Nathans *et al*., 1986)
54. Human blue opsin (Nathans *et al*., 1986)

55. Odorant receptor F3 (Buck and Axel, 1991)
56. Odorant receptor F5 (Buck and Axel, 1991)
57. Odorant receptor F6 (Buck and Axel, 1991)
58. Odorant receptor F12 (Buck and Axel, 1991)
59. Odorant receptor I3 (Buck and Axel, 1991)
60. Odorant receptor I7 (Buck and Axel, 1991)
61. Odorant receptor I8 (Buck and Axel, 1991)
62. Odorant receptor I9 (Buck and Axel, 1991)
63. Odorant receptor I14 (Buck and Axel, 1991)
64. Odorant receptor I15 (Buck and Axel, 1991)

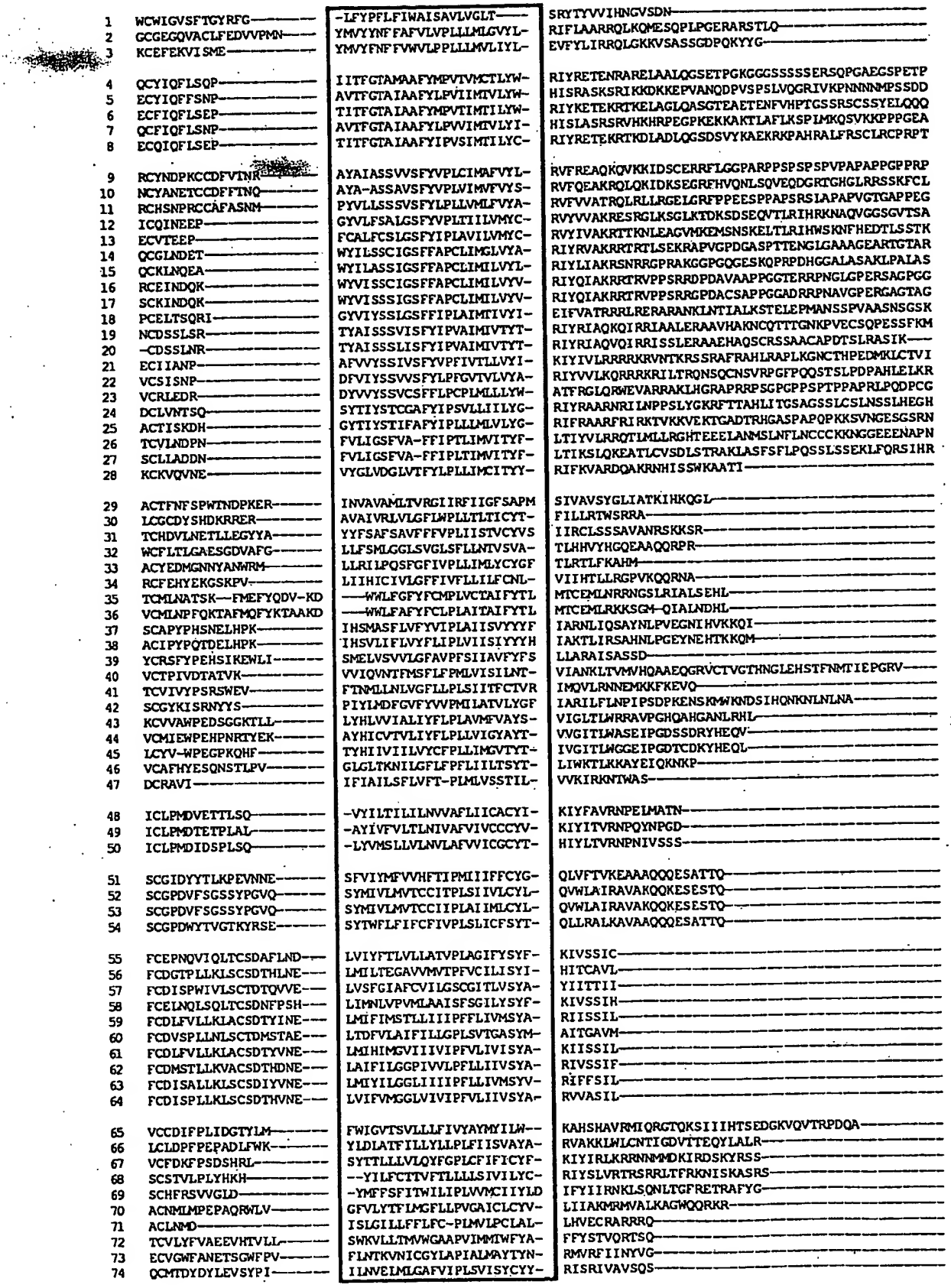
65. Human cannabinoid receptor (Matsuda *et al*., 1990)
66. Mouse Glucocorticoid-induced receptor (Harrigan *et al*., 1991)
67. Rat FC5R (Eva *et al*., 1990)
68. Human endothelial cell GPR (Hla and Maciag, 1990)
69. Rat testis G-protein coupled receptor 1 (Meyerhof *et al*. 1991a)
70. Rat RGHJP (Meyerhof, DNA and Cell Biology, in press, 1991b).
71. Human thoracic aorta GPR (Ross *et al*., 1990)
72. Cytomegalovirus (Human) GPR, US33 (Chee *et al*., 1990)
73. Cytomegalovirus (Human) GPR, US27 (Chee *et al*., 1990)
74. Cytomegalovirus (Human) GPR, US28 (Chee *et al*., 1990)

```
 1                                                                                                                MGLLDGNPANET
 2                                                                                                                  MSIMGSW
 3                                                                                                                MPPAISAFQA

 4                                                                                                             MNTSAPPAVSPNITVLAPGKGPWQ
 5                                                                                                           MNNSTNSSNNSLALTSPYKTFE
 6                                                                                  MTLHNNSTTSSPLFPNISSSWIHSPSDAGLPPGTVTHFGSYNVSRAAGNFSSNDGTTDDPLGGHTVWQ
 7                                                                                                             MANFTPVNGSSGNQSVRLVTSSSHNRYETVE
 8                                                                                                             MEGDSYHNATTVNGTPVNHQPLERHRLWE

 9                                                                                                  MGAGVLVLGASEPGNLSSAAPLPDGAATAARLLVPASPPASLLPPASESPEPLSQQW
10                                                                                                    MAPWPHENSSLAPWPDLPTLAPNTANTSGLPGVPWE
11                                                                                                    MGQPGNGSAFLLAPNRSHAPDHDVTQQRDEVW
12                                                                                                          MVFLSGNASDSSNCTHPPPPVNISK
13                                                                                                  MNPDLDTGHNTSAPAHWGELKDDNFTGPNQTSSNSTLPQLDVTR
14                                                                                                MASPALAAALAVAAAAGPNASGAGERGSGGVANASGASWGPPRGQYSAGA
15                                                                                                                MDHQDPYSVQA
16                                                                                                    MGSLQPDAGNASWNGTEAPGGGARATPYSLQV
17                                                                                                    MGSLQPDAGNSSWNGTEAPGGGTRATPYSLQV
18                                                                                  MPSADQILFVNVTTTVAAAALTAAAAVSTTKSGNAARGYTDSDDDAGMGTEAVANISGSLVEGLTTVTAALS-(35)
19                                                                                                          MRTLNTSAMDGTGLVVERDFSV
20                                                                                                  MLPPGSNGTAYPGQFALYQQLAQGNAVGGSAGAPPLGPS
21                                                                                                    MDPLNLSWYDDDLERQNWSRPFNGSDGKADRPH
22                                                                                                      MASLSQLSSHLNSTCGAENSTGASQARPH
23                                                                                                    MGNRSTADADGLLAGRGPAAGASAGASAGLAGQ
24                                                                                                  MSPLNQSAEGLPQEASNRSLNATETSEAWNPRTLQAL
25                                                                                                    MDVLSPGQGNNTTSPPAPFETGGNTTGISDVTVSYQ
26                                                                                                MVNLGNAVSLLMHIGLLVWQFDISISPVAGIVTDTFNSSDGGRLFQFPDGV
27                                                                                  MEILCEDNISLSSIPNSLMQLGDGPRLYHNDFNSRDANTSEASNWTIDAENRTNLSCEGYLPPTCLSILHLQE
28                                                                                                              MAPNGTASSFCLDSTACK

29                                                                                                              METNSSLPTNISGGTPAVSAGYLFLD
30                                                                                                            MNSFNYTTPDYGHYDDKDTLDLNTPVDKTSNTLRVP
31                                                                  MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLDPRSFLLLRNPNDKYEPFWEDEEKNESGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWL
32                                                                                                              MWPNGSSLGPCFRPTNITLEERR
33                                                                                                          MESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPESLEIN
34                                                                                                              MELNSSSRVDSEFRYT
35                                                                                                  METFWLRLSFWVALVGGVISDNPESYSTNLSIHVDSVATFHGTELSFVVTTHQPTNLALPSNGSMHNYCPQQTKITSAFK
36                                                                  MQSSASRCGRALVALLLACGLLGVWGEKRGFPPAQATPSLLGTKEVMTPPTKTSWTRGSNSSLMRFRTAEVTKGGRVAGVPPRSFPPPCQRKIEINKTFK
37                                                                                                            MMAPNNCSHLNLDVDPFLSCNDTFNQSLSPPKMDNWFHPGF
38                                                                                                          MPPRSLPNLSLPTEASESELEPEVWENDFLPDSDGTTAELVIR
39                                                                                                        MDLHLFDYAEPGNFSDISWPCNSSDCIVVDTVMCPNMPNKSVLL
40                                                                                                  MHLNSSVPQGTPGEPDAQPFSGPQSEMEATFLALSLSNGSGNTSESDTAGPNSDLDVNTDIYS
41                                                                                                              MFNITTQALGSAHNGTSFEVNCPDTEWWSWLN
42                                                                                                              MENDTVSEMNQTELQPQAAVALEYQVVT
43                                                                                                            MGTCDIVTEANISSGPESNTTGITAFSMPSWQ
44                                                                                                            MDNVLPMDSDLFPNISTNTSESNQFVQPTWQ
45                                                                                  MASVPRGENWTDGTVEVGTHTGNLSSALGVTEWLALQAGNFSSALGLPATTQAPSQVRANLTNQFVQPSWR
46                                                                                                              MILNSSTEDGIKRIQDDCPKAGRHNYIFI
47                                                                                                            MDGSNVTSFVVEEPTNISTGRNASVGNAHRQIP

48  MKQRFSPLQLLKLLLLLQAPLPRALRRLCPEPCN-(248)-LPTKELNFSHSISENFSKQCESTVRKSELSGWDYEYGFCLPKTPRCAPEPDAFNPCEDIMG
49  MRPADLLQLVLLLDLPRDLGGMGCSSPPCECHQE-(318)-YVFFEEQEDEIIGFGQELKNPQEETLQAFDSHYDYTICGDSEDMVCTPKSDEFNPCEDIMG
50  MALLLVSLLAFLSLGSGCHHRICHCSNRVFLCQE-(266)-VDYMTQARGQRSSLAEDNESSYSRGFDMTYTEFDYDLCNEVVDVTCSPKPDAFNPCEDIMG

51                                                                                                          MNGTEGPNFYVPFSNATGVVRSPFEYPQYYLAEPWQF
52                                                                                                  MAQQWSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWVYHLTSVW
53                                                                                                  MAQQWSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWVYHLTSVW
54                                                                                                          MRKMSEEEFYLFKNISSVGPWDGPQYHAIPVWAFYL

55                                                                                                              MDSSNRTRVSEFLLLGFVENKDLQP
56                                                                                                              MSSTNQSSVTEFLLLGLSRQPQQQQ
57                                                                                                            MAWSTGQNLSTPGPFILLGFPGPRSMRI
58                                                                                                            MESGNSTRRFSSFFLLGFTENPQLHF
59                                                                                                              MNNQTFITQFLLLGLPIPEEHQH
60                                                                                                            MERRNHSGRVSEFVLLGLPAPAPLRV
61                                                                                                              MNNKTVITHFLLLGLPIPPEHQQ
62                                                                                                            MTRRNQTAISQFFLLGLPFPPEYQH
63                                                                                                            MTGNNQTLILEFLLLGLPIPSEYHL
64                                                                                                            MTEENQTVISQFLLLFLPIPSEHQH

65  MKSILDGLADTTFRTITTDLLYVGSNDIQYEDIK-(21)-SPFQEKMTAGDNSPLVPAGDTTNITEFYNKSLSSFKENEENIQCGENFMDMECFMILNPSQQ
66                                                                                                  KVPPVLLLFLLSSVRATEQPQVVTEHPSWEAALTGPNASSHFWANYTFSDWQNFVGRRRYGAESQNPTV
67                                                                                                              MNSTLSFRVENYSVHYNVSENSPFLAFENDDCHLPLAV
68                                                                                                  MGPTSVPLVKAHRSSVSDYVNYDIIVRHYNYTGKLNISADKENSIK
69                                                                                                              MKANNTTTSALWLQ
70                                                                                                MFPNGTAPSPTSSPSSSPGGCGEGVCSRGPGSGAADGMEEPGRNSSQNGTLSEGQGS
71                                                                                                  MAGNCSWEAHSTNQNKMCPGMSEALELYSRGFLTIEQIATLPPPA
72                                                                                                                MTGPLFAIR
73                                                                                                          MTTSTNNQTLTQVSNMTNHTLNSTEIYQLFEYTR
74                                                                                                          MTPTTTTAELTTEFDYDEDATPCVFTDVLNQSK
```

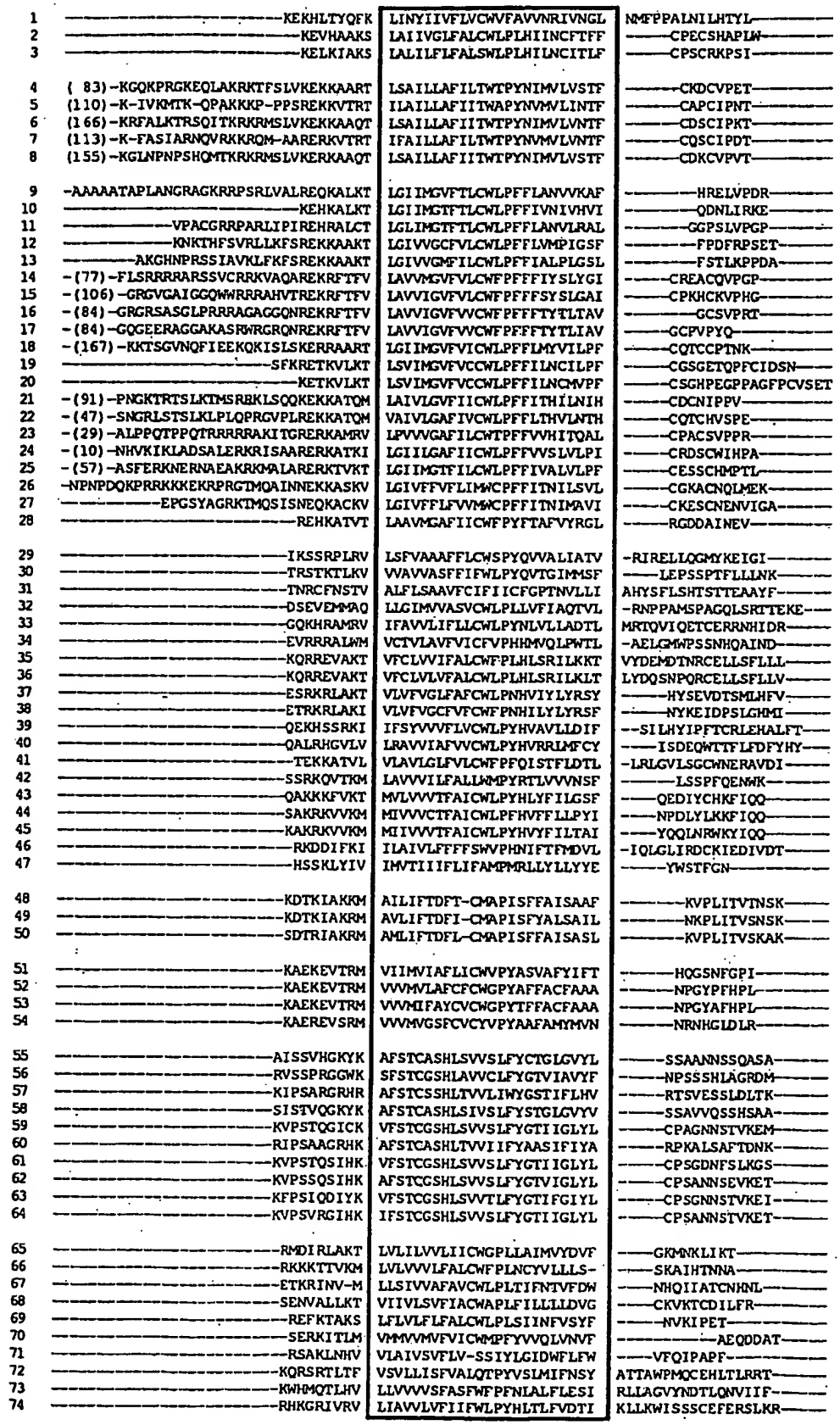

1
2


3
4

5



6



7

1	SVSHGFWASVTFIYNNPLM-WRYF	GAKILTVFTFFGYFTDVQKKLEKNKNNNNPSPYSSSRGTSGKTMGGHPTGDDVQCSSDMEQCSLERHPNMV- (63)
2	LMYLTIVLSHTNSVVNPFI-YAYR	IREFRQTFRKIIRSHVLRRREPFKAGGTSARALAAHGSDGEQISLRLNGHPPGVWANGSAPHPERRPNGYT- (50)
3	LMYIAIFLTHGNSAMNPIV-YAFR	IQKFRVTFLKIWNDHFRCQPTPPVDEDPPEEAPHD
4	LWELGYWLCYVNSTINPMC-YALC	NKAFRDTFRLLLLLCRWDKRRWRKIPKRPGSVHRTPSRQC
5	VWTIGYWLCYINSTINPAC-YALC	NATFKKTFKHLLMCHYKNIGATR
6	YWNLGYWLCYINSTVNPVC-YALC	NKTFRTTFKTLLLCQCDKRKRRKQQYQQRQSVIFHKRVPEQAL
7	VWSIGYWLCYVNSTINPAC-YALC	NATFKKTFRHLLLCQRYNIGTAR
8	LWHLGYWLCYINSTVNPIC-YALC	NRTFRKTFKMLLLCRWKKKKVEEKLYWQGNSKLP
9	LFVFFNWLGYANSAFNPII-YCRS	PDFRKAFQGLLCCARRAARRRHATHGDRPRASGCLARPGPPSPGAASDDDDDDVVGATPPARLLEPWAGCN- (25)
10	VYILLNWIGYVNSGFNPLI-YCRS	PDFRIAFQELLCLRRSSLKAYGNGYSSNGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNID- (13)
11	AFLALNWLGYANSAFNPLI-YCRS	PDFRSAFRRLLCRCGRRLPPEPCAAARPALFPSGVPAAESSPAQPRLCQRLDG
12	VFKIAFWLGYLNSCINPII-YPCS	SQEFKKAFQNVLRIQCLRRKQSSKHTLGYTLHAPSHVLEGQHKDLVRIPVGSAETFYKISKTDGVCEWKIF- (66)
13	VFKVVFWLGYFNSCLNPII-YPCS	SKEFKRAFMRILGCQCRGGRRRRRRRRLGACAYTYRPWTRGGSLERSQSRKDSLDDSGSCMSGQKRTLPSA- (93)
14	LFKFFFWIGYCNSSLNPVI-YTVF	NQDFRPSFKHILFRRRRRGFRQ
15	LFQFFFWIGYCNSSLNPVI-YTIF	NQDFRRAFRRILCRPWTQTAW
16	LFKFFFWFGYCNSSLNPVI-YTIF	NHDFRRAFKKILCRGDRKRIV
17	LFNFFFWFGYCNSSLNPVI-YTIF	NHDFRRAFKKILCRGDRKRIV
18	FKNFITWLGYINSGLNPVI-YTIF	NLDYRRAFKRLLGLN
19	TFDVFVWFGWANSSLNPII-YA-F	NADFRKAFSTLLGCYRLCPATNNAIETVSINNNGAAMFSSHHEPRGSISKECNLVYLIPHAVGSSEDLKKE- (42)
20	TFDVFVWFGWANSSLNPVI-YA-F	NADFQKVFAQLLGCSHFCSRTPVETVNISNELISYNQDIVFHKEIAAAYIHMMPNAVTPGNREVDNDEEEG- (45)
21	LYSAFTWLGYVNSAVNPII-YTTF	NIEFRKAFLKILHC
22	LYSATTWLGYVNSALNPVI-YTTF	NIEFRKAFLKILSC
23	LVSAVTWLGYVNSALNPVI-YTVF	NAEFRNVFRKALRACC
24	LFDFFTWLGYLNSLINPII-YTVF	NEEFRQAFQKIVPFRKAS
25	LGAIINWLCVINSLLNPVI-YAYF	NKDFQNAFKKIIKCNFCRQ
26	LLNVFVWIGYVCSGINPVI-YTLF	NKIYRRAFSKYLRCDYKPDKKPPVRQIPRVAATALSGRELNVNIYRHTNERVARKANDPEPGIENQVENLE- (16)
27	LLNVFVWIGYLSSAVNPLV-YTLF	NKTYRSAFSRYLQCQYKENRKPLQLILVNTIPALAYKSSQLQVGQKKNSQEDAEQTVDDCSMVTLGKQQSE- (17)
28	LEAIVLWLGYANSALNPIL-YAAL	NRDFRTGYQQLFCCRLANRNSHKTSLRSNASQLSRTQSREPRQQEEKPLKLQVWSGTEVTAPQGATDR
29	AVDVTSALAFFNSCLNPML-YVFM	GQDFRERLIHALPASLERALTEDSTQTSDTAINSTLPSAEVALQAK
30	LDSLCVSFAYINCCINPII-YVVA	GQGQFQGRLRKSLPSLLRNVLTEESVVRESKSFTRSTVDTMAQKTQAV
31	AYLLCVCVSSISSCIDPLI-YYYA	SSECQRYVYSILCCKESSDPSSYNSSGQLMASKMDTCSSNLNNSIYKKLLT
32	-LLIYLRVATWNQILDPWV-YILF	RRAVLRRLQPRLSTRPRSLSLQPQLTQRSGLQ
33	ALDATEILGILHSCLNPLI-YAFI	GQKFRHGLLKILAIHGLISKDSLPKDSRPSFVGSSSGHTSTTL
34	AHQVTLCLLSTNCVLDPVI-YCFL	TKKFRKHLSEKLNIMRSSQKCSRVTTDTGTEMAIPINHTPVNPIKN
35	MDYIGINLATMNSCINPIALYFVS	KKFKNCFQSCLCCCCYQSKSLMTSVPMQGTSIQWKNHEQNNHNTERSSHKDSIN
36	LDYIGINMASLNSCINPIALYLVS	KRFKNCFKSCLCCWCQTFEEKQSLEEKQSCLKFKANDHGYDNFRSSNKYSSS
37	TSICARLIAPTNSCVNPFALYLLS	KSFRQFNTQLLCCQPGLMNRSHSTGRSTTCMTSFKSTNPSATFSLINRNICHEGYV
38	VTLVARVLSFSNSCVNPFALYLLS	ESFRKHFSNQLCCGQKSYPERSTSYLLSSSAVRMTSLKSNAKNVVTNSVLLNGHSTKQEIAL
39	ALHVTQCLSLVHCCVNPVL-YSFI	NRNYRYELMKAFIFKYSAKTGLTKLIDASRVSETEYSALEQNAK
40	FYMLTNALFYVSSAINPIL-YNLV	SANFRQVFLSTLACLCPGWRHRRKKRPTFSRKPNSMSSNHAFSTSATRETLY
41	VTQISSYVAYSNSCLNPLV-YVIV	GKRFRKKSREVYQAICRKGGCMGESVQMENSMGTLRTSISVDRQIHKLQDWAGNKQ
42	-LLKCRICIYLNSAINPVI-YNLM	SQKRFAAFRKLCNCKQKPTEKAANYSVALNYSVIKESDRFSTELEDITVTDTYVSTTKVSFDDTCLASEN
43	VYLALFWLAMSSTMYNPII-YCCL	NHRFRSGFRLAFRCCPWVTPTKEDKLELTPTTSLSTRVNRCHTKETLFMAGDTAPSEATSGEAGRPQDGSG- (17)
44	VYLASMWLAMSSTMYNPII-YCCL	NDRFRLGFKHAFRCCPFISAGDYEGLEMKSTRYLQT-QSSVYKVSRLETTISTVVGAHEEEPEEGPKATPS- (29)
45	VYLASFWLAMSSTMYNPII-YCCL	NKRFRAGFKRAFRWCPFIQVSSYDELELKTTRFHPTRQSSLYTVSRMESVTVLFDPNDGDPTKSSRKKRAV- (34)
46	AMPITICLAYFQQNLNPLF-YGFL	GKKFKKYFLQLLKYIPPKAKSHSNLSTKMSTLSYRPSEQGNSSTKKPAPCIEVE
47	LHHISLLFSTINSSANPFI-YFFV	GSSKKKRFKESLKVVLTRAFKDEMQPRRQKDNC-NTVTVETVV
48	VLLVLFYPI--NSCANPFL-YAIF	TKTFQRDFFLLLSKFGCCKRRADIYRRKDFSAYTSNCKNGFTGSNKPSQSTLKLSTLHCQGTALLDKTRYTEC
49	ILLVLFYPL--NSCANPFL-YAIF	TKAFQRDVFILLSKFGICKRQAQAYRGQRVPPKNSTDIQVQKVTHDMRQGALNMEDVVELIENSHLTPKKQ- (12)
50	ILLVLFHPI--NSCANPFL-YAIF	TKNFRRDFFILLSKCGCYEMQAQIYRTETSSTVHNTHPRNGHCSSAPRVTSGSSTYILVPLSHLAQN
51	FMTIPAFFAKSAAIYNPVI-YIMM	NKQFRNCMLTTICCGKNPLGDDEASATVSKTETSQVAPA
52	MAALPAFFAKSATIYNPVI-YVFM	NRQFRNCILQLFGKKVDDGSELSSASKTEVSSVSSVSPA
53	MAALPAYFAKSATIYNPVI-YVFM	NRQFRNCILQLFGKKVDDGSELSSASKTEVSSVSSVSPA
54	LVTIPSFFSKSACIYNPII-YCFM	NKQFQACIMKMVCGKAMTDESDTCSSQKTEVSTVSSTQVGPN
55	--TASVMYTVVTPMVNPFI-YSL-	RNKDVKSVLKKTLCEEVIRSPPSLLHFFLVLCHLPCFIFCY
56	--AAAVMYAVVTPMLNPFI-YSL-	RNSDMKAALRKVLAMRFPSKQ
57	--AITVLNTIVTPVLNPFI-YTL-	RNKDVKEALRRTVKGK
58	--SASVMYTVVTPMLNPFI-YSL-	RNKDVKRALERLLEGNCKVHHWTG
59	--VMAMMYTVVTPMLNPFI-YSL-	RNRDMKRALIRVICSMKITL
60	--LVSVLYAVIVPLFNPII-YCL-	RNQDVKRALRRTLHLAQDQEANTNKGSKIG
61	--AMAMMYTVVTPLMNPFI-YSL-	RNRDMKQALIRVTCSKKISLPW
62	--VMSLMYTMVTPMLNPFI-YSL-	RNRDIKDALEKIMCKKQIPSFL
63	--AMAMMYTVVTPMLNPFI-YSL-	RNRDMKRALIRVICTKKISL
64	--VMAMMYTVVTPMLNPFI-YSL-	RNRDMKEALIRVLCKKKITFCL
65	VFAFCSMLCLLNSTVNPII-YALR	SKDLRHAFRSMFPSCEGTAQPLDNSMGDSDCLHKHANNTASMHRAAESCIKSTVKIAKVTMSVSTDTSAEAL
66	LYFAFHWFAMSSTCYNPFI-YCWL	NENFRVELKALLSMCQRPPKPEDRLPSPVPSFRVAWTEKSHGRRAPLPNHHLPSSQIQSGKTDLSSVEPVVAMS
67	LFLLCHLTAMISTCVNPIF-YGFL	NKNFQRDLQFFFNFCDFRSRDGRTTRL
68	AEYFLV-LAVLNSGTNPII-YTLT	NKEMRRAFIRIMSCCKCPSGDSAGKFKRPIIAGMEFSRSKSDNSSHPQKDEGDNPETIMSSGNVNSSS
69	AMCLGILLSHANSMMNPIV-YACK	KKFKETYFVILRACRLCQTSDSLDSNLEQTTE
70	VSQLSVILGYANSCANPIL-YGFL	SDNFKRSFQRILCLSWMDNAAEEPVDYYATALKSRAYSVEDFQPENLESGGVFRNGTCASRISTL
71	PEYVTDLCICINSSAKPIV-YFLA	GRDKSQRLWEPLRVVFQRALRDGAEPGDAASSTPNTVTMEMQCPSGNAS
72	IGTLARVVPHLHCLINPIL-YALL	GHDFLQRMRQCFRGQLLDRAFLRSQQNQRA
73	CLYVGQFLAYVRACLNPGI-YILV	GTQMRKDMWTTLRVFACCCVKQEIPYQDIDI
74	ALILTESLAFCHCCLNPLL-YVFV	GTKFRKNYTVCWPSFASDSFPAMYPGTTA

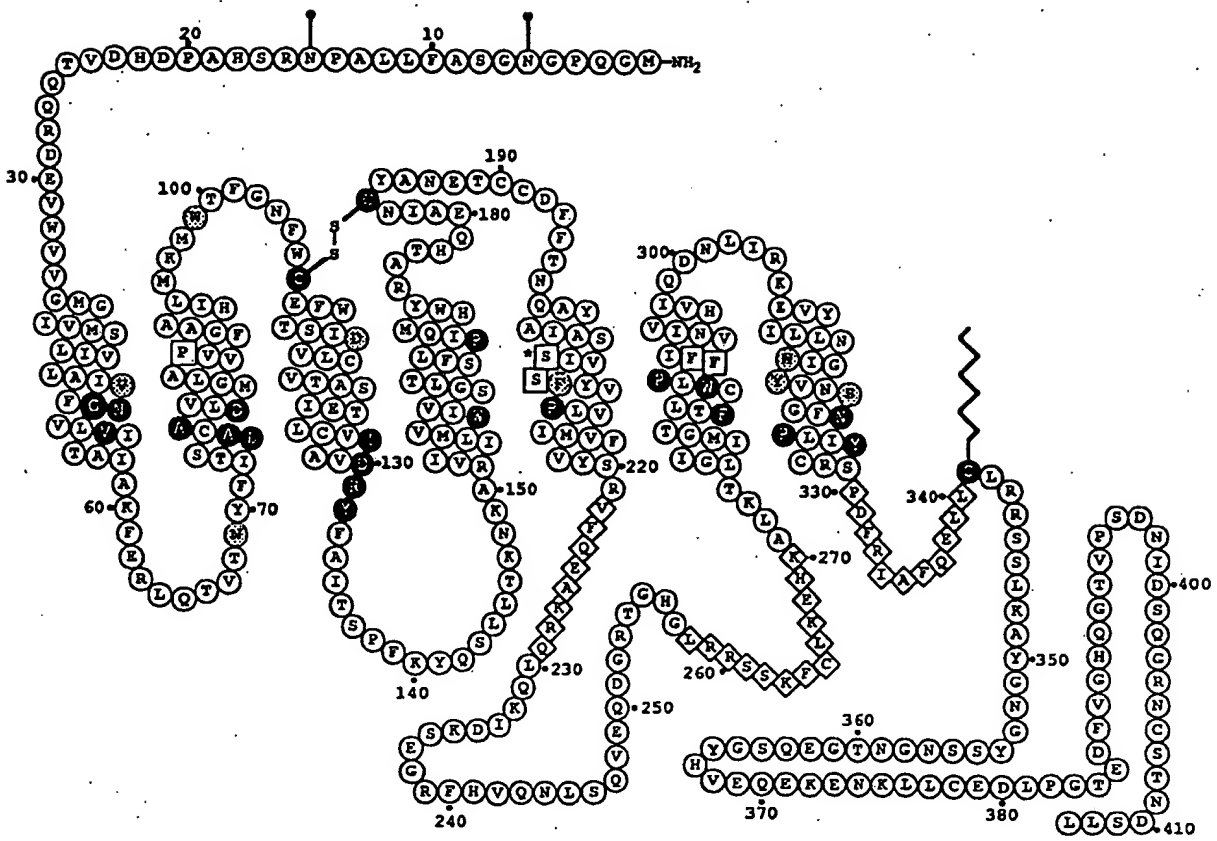


FIG. 3. Model of the human β_2-adrenergic receptor. Amino acid residues in black are conserved in nearly all of the GPRs. Stippled residues are conserved in cationic amine receptors. Boxed residues are conserved in all catecholamine receptors. The asterisk denotes the serine conserved in the serotonin receptors. Residues in diamonds are residues believed to be involved in G-protein coupling. Glycosylated asparagines (N-6 and N-15) within the amino terminus are indicated. Cys^{341} of the carboxyl terminus is known to be palmitoylated. Protein kinase A phosphorylation sites are indicated by arrowheads.

LIGAND BINDING DOMAINS

Our understanding of the structure of the binding site of the GPRs and of which residues actually interact with agonists and antagonists is rapidly evolving. The ligand binding sites of rhodopsin and of the adrenergic and muscarinic receptors have been partly delineated through biochemical and molecular biological approaches (for review, see Applebury and Hargrave, 1986; O'Dowd *et al.*, 1989b; Strader *et al.*, 1989b; Venter *et al.*, 1989; Hulme *et al.*, 1990). For most of the GPRs, with the possible exception of the glycoprotein hormone receptors, the ligand binding pocket appears to be created by the membrane-spanning regions.

As none of the GPRs have yet been crystallized, modeling of the three-dimensional array of the helices is based on the structure of bacteriorhodopsin, which has recently been resolved at high resolution (Henderson *et al.*, 1990). The transmembrane domains appear to form a hydrophilic pocket for ligand binding surrounded by hydrophobic residues (Strader *et al.*, 1989b; Venter *et al.*, 1989; Hulme *et al.*, 1990). The putative arrangement of the residues around the ligand binding site have been analyzed through helical wheel modeling. The α-helices contains 3.6 residues per helical turn. When the assortment of residues around the helix is predicted for the muscarinic receptors (Hulme *et al.*, 1990), adrenergic receptors (Strader *et al.*, 1989b; Venter *et al.*, 1989), and many other GPRs (Donnelly *et al.*, 1989), the domains contain a predominance of hydrophobic residues on one side and hydrophilic on the other. The hydrophilic side of each helix is postulated to face inwards and form the polar ligand binding site. Recently, computer-generated models for ligand–receptor interactions have been developed (Findlay and Eliopoulos, 1990; Henderson *et al.*, 1990; Dahl *et al.*, 1990; Hibert *et al.*, 1991).

The presence of a ligand binding pocket for the chromophore retinal deep within the transmembrane α-helices of rhodopsin was suggested by cross-linking and fluorescent energy transfer studies (Hargrave *et al.*, 1982; Thomas and Stryer, 1982). Retinal forms a Shiff base linkage with Lys^{296} in TM 7 (Thomas and Stryer, 1982). The Glu^{113} of

rhodopsin in TM 3 has been proposed as a counterion that interacts with the protonated Shiff base retinal, although mutagenesis studies have been inconclusive (Sakmar *et al.*, 1989; Zhukovsky and Oprian, 1989).

A variety of approaches support the existence of a similar intrahelical binding site in the cationic amine receptors. The ligand binding site of the adrenergic receptors has been investigated by photoaffinity labeling (Bar-Sinai *et al.*, 1986; Dohlman *et al.*, 1988), fluorescence emission spectroscopy (Tota and Strader, 1990), deletion mutants (Dixon *et al.*, 1987a,b), site-directed mutagenesis (Chung *et al.*, 1988; Dixon *et al.*, 1988; Strader *et al.*, 1988; Fraser, 1989; Strader *et al.*, 1989a,b; Wang *et al.*, 1991), and receptor chimeras (Kobilka *et al.*, 1988). As was the case for the visual pigments, the transmembrane domains are necessary for ligand binding and confer ligand specificity, while the hydrophilic extracellular and intracellular domains are not directly involved in ligand binding (Dixon *et al.*, 1981a,b). In both the α- and β-adrenergic receptors (Strader *et al.*, 1988; Wang *et al.*, 1991) as well as the m_1 muscarinic receptor (Fraser *et al.*, 1989), site-directed mutagenesis has demonstrated that the TM 3 aspartate (Asp^{113} in the β-adrenergic receptor; see Fig. 3) is critical for wild-type agonist and antagonist binding. The cationic amines, which include epinephrine, norepinephrine, dopamine, serotonin, and acetylcholine, all contain a positively charged amine head group which most likely interacts with the conserved TM 3 aspartate found in these receptors.

Mutagenesis studies have suggested that particular residues conserved within receptor subclasses can contribute to agonist specificity. Two conserved serines in TM 5 (Ser^{204} and Ser^{207} in the β-adrenergic receptor) have been implicated in forming hydrogen bonds with the *meta*- and *para*-hydroxyl groups of adrenergic agonists. Replacement of either serine by alanine reduces agonist binding to the same degree as removing the corresponding hydroxyl group from the ligand (Strader *et al.*, 1989a). Recent mutagenesis studies of the α_2-adrenergic receptor suggest that Ser^{204} (corresponding in position to Ser^{207} of the β-adrenergic receptor) binds in an analogous fashion to the *para*-hydroxyl group of adrenergic ligands (Wang *et al.*, 1991). Two corresponding serine residues are found in TM 5 in all the dopamine receptors and a single conserved serine residue in TM 5 of the serotonin receptors (Fig. 2). The similarity of ligand structure and receptor sequence suggests that these TM 5 serines may also hydrogen bond with the aromatic hydroxyl groups of their respective agonists. This hypothesis is supported by computer modeling of the ligand–receptor interaction (Hibert *et al.*, 1991). The muscarinic receptors all contain a conserved asparagine in TM 6, not found in any other receptor subclass, which has been proposed to interact with the ester group of acetylcholine (Hibert *et al.*, 1991). Conserved TM 6 and/or TM 7 aromatic residues (*e.g.*, Phe^{290} and Tyr^{326} in the β-adrenergic receptor) may interact with the aryl ring of serotonergic and adrenergic ligands (Dixon *et al.*, 1988; Hibert *et al.*, 1991).

The ligands for the glycoprotein hormone receptors, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and lutenizing hormone/chorionic gonadotropin (LH/CG), are much larger than the ligands for the other GPRs. Presumably because of the large size of the ligands, this receptor subclass has evolved a distinct structure containing an extremely long first cytoplasmic domain encompassing the high-affinity hormone binding site. This glycosylated extracellular domain is rich in cysteine residues that may form disulfide bridges and help maintain the three-dimensional structure of the proteins (Sprengel *et al.*, 1990). The large amino-terminal extracellular domain of these receptors contains multiple leucine-rich repeats that identify these GPRs as members of a second gene family, that of the leucine-rich glycoprotein family (Takahashi *et al.*, 1985; Krusius and Ruoslahti, 1986). The extracellular location of a hormone binding site is supported by chimera studies (Moyle *et al.*, 1991; Nagayama *et al.*, 1991b) and, in the case of the LH/CG receptor, by the secretion of a soluble hormone binding protein generated by alternative splicing which encompasses only the amino terminus (Loosfelt *et al.*, 1989; Tsai-Morris *et al.*, 1990). Short regions of the amino terminus of TSH and LH/CG are necessary for high-affinity hormone binding (Wadsworth *et al.*, 1990; Nagayama *et al.*, 1991a,b). In contrast, β-adrenergic receptor ligand binding is not dependent on the amino-terminal extracellular domain (Dixon *et al.*, 1987b).

Recently the binding and activation of an LH/CG receptor construct in which virtually the entire extracellular amino terminus has been deleted was investigated (Ji and Ji, 1991b). The finding that CG can bind to the seven transmembrane components of the receptor, albeit with lower affinity, in the absence of the extracellular amino terminus suggests that this receptor may contain both a high-affinity binding site extracellularly and a low-affinity site within the transmembrane domains. CG binding to this low-affinity receptor mutant was capable of stimulating cAMP production. Possibly the high-affinity extracellular binding site serves to capture the hormone and present it to the intramembranous binding pocket for signal transduction.

INTRACELLULAR COUPLING

The GPRs are coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters (Johnson and Dhanasekaran, 1989; Birnbaumer *et al.*, 1990). The G-proteins, which associate with GPRs at the intracellular face of the plasma membrane, are composed of relatively invariant β- and γ-subunits and a variable α-subunit (α_s, α_i, α_o) for which the G-protein is named (G_s, G_i, G_o). By a process not yet understood, GPR agonist binding catalyzes the exchange of GTP for GDP on the α-subunit (G-protein "activation"), resulting in its dissociation and stimulation of one (or more) of the various signal-transducing enzymes and channels. The different G-protein α-subunits preferentially stimulate particular effectors. The specificity of signal transduction may be determined, therefore, by the specificity of G-protein coupling. Some GPR residues or regions which are necessary for efficient signal transduction can be postulated to interact

with conserved G-protein motifs. In addition, certain short amino acid stretches of the receptors which are necessary for G-protein coupling also determine the specificity of the G-protein interactions.

Three types of studies investigating the relationship between receptor structure and G-protein affinity have been performed. Deletion and site-directed mutagenesis studies implicate receptor regions and amino acid residues that are necessary for efficient G-protein coupling. Synthetic peptide competition studies suggest which oligopeptide domains may directly interact with the G-proteins. Chimera experiments delineate the receptor regions that determine the specificity of G-protein coupling. Certain general principles arise from these multifaceted investigations. All of the intracellular domains are implicated in efficient G-protein coupling of various receptors. Short stretches of the membrane proximal regions of the third cytoplasmic loop and possibly the carboxyl terminus appear particularly critical in determining the specificity of G-protein coupling for many receptors.

Single residue mutations in the cytoplasmic loops of the β-adrenergic receptor reduce signal transduction (Dixon *et al.*, 1988; O'Dowd *et al.*, 1988). Site-directed mutagenesis of a conserved proline in the second intracellular loop to threonine, for example, caused no change in agonist binding but a ~35% reduction in adenylate cyclase stimulation (O'Dowd *et al.*, 1988). Site-directed mutagenesis has identified particular charged residues in the membrane proximal regions of the second and third intracellular loops which contribute to efficient G-protein coupling. Mutation of the highly conserved aspartate adjacent to TM 3 in the second intracellular loop of the β-adrenergic receptor (Asp^{130} which is in the "DRY" sequence) gives rise to a receptor with high-affinity ligand binding but reduced or absent G-protein coupling (Dixon *et al.*, 1988; Fraser *et al.*, 1988). Similar results have been obtained for the muscarinic m_1 and the α_{2A}-adrenergic receptors (Fraser *et al.*, 1988, 1989; Wang *et al.*, 1991). The corresponding glutamate of rhodopsin is similarly implicated as interacting with transducin (Franke *et al.*, 1990). Another residue needed for transducin activation by rhodopsin is the lysine located in the distal third intracellular loop, Lys^{248}. Mutation of this lysine to leucine results in a complete loss of signal transduction (Franke *et al.*, 1988). Mutation or deletion of histamine at the corresponding position in the β-adrenergic receptor reduced, although did not abolish, adenylate cyclase stimulation (O'Dowd *et al.*, 1988).

The TM 2 aspartate (Asp^{79} of the β-adrenergic receptor, see Fig. 3), which is conserved in virtually all GPRs, is necessary for wild-type agonist binding and G-protein activation in many GPRs studied (Chung *et al.*, 1988; Strader *et al.*, 1988; Fraser *et al.*, 1989; Wang *et al.*, 1991; Ji and Ji, 1991a). In the α_2-adrenergic and dopamine D_2 receptors, this aspartate is essential for modulation of receptor coupling by Na^+ and H^+, possibly due to allosteric modulation of receptor conformation (Horstman *et al.*, 1990; Neve, 1991; Neve *et al.*, 1991). Another transmembrane residue, the TM 6 cysteine found in most GPRs, has been implicated in β-adrenergic receptor signal transduction (Fraser, 1989).

Deletion studies of the β_2-adrenergic receptor have indicated that the membrane proximal regions of the third cytoplasmic loop (residues 222–229 and 258–270, see Fig. 3) are necessary for signal transduction (Strader *et al.*, 1987a; O'Dowd *et al.*, 1988). In the α_1-adrenergic receptor, deletion of seven amino acids of the third intracellular loop proximal to TM 7 caused a marked reduction in coupling to phospholipase C (Cotecchia *et al.*, 1990).

Deletions of carboxy-terminal residues adjacent to TM 7 produce mutant rhodopsin or β_2-adrenergic receptors with diminished ability to activate G-proteins (O'Dowd *et al.*, 1988; Franke *et al.*, 1990). Mutation of a palmitoylated carboxy-terminal Cys^{341} to glycine markedly reduced agonist stimulation of adenylate cyclase of the β_2-adrenergic receptor (O'Dowd *et al.*, 1989a). The palmitoylated cysteine is predicted to anchor the carboxyl terminus to the membrane, producing a fourth cytoplasmic loop (see Fig. 3). Membrane anchorage may optimally position carboxy-terminal residues for G-protein interaction (Ovchinnikov *et al.*, 1988; O'Dowd *et al.*, 1989a). Regions of the carboxyl terminus and third cytoplasmic loop, adjacent to the transmembrane domains, may form clustered amphipathic α-helices (Strader *et al.*, 1987a; Higashijima *et al.*, 1988; Strader *et al.*, 1989b; Palm *et al.*, 1990). These helices, along with charged intracellular residues of the second and third intracellular loops (*i.e.*, DRY), may cooperatively interact to efficiently bind and activate G-proteins.

The activation of G-proteins by amphipathic α-helices is supported by experiments in which the G proteins G_i and G_o have been directly activated by mastoparan and other small peptides which form amphipathic α-helices at the inner surface of the cytoplasmic membrane (Higashijima *et al.*, 1988, 1990). Furthermore, direct activation of G_s has been demonstrated for synthetic peptides representing the third intracellular loop sequences adjacent to TM 5 and TM 6 of the β_2-adrenergic receptor (Cheung *et al.*, 1991), and by a peptide representing the intracellular third loop sequence proximal to TM 6 of the avian β-adrenergic receptor (Palm *et al.*, 1989; Munch *et al.*, 1991).

Peptide competition experiments, in which short synthetic peptides competitively bind to G-proteins but do not activate them, have been useful in mapping GPR regions that are likely to contact the G-proteins. Receptor uncoupling following mutagenesis or deletion of receptor segments may be due either to loss of G-protein contacts or to altered tertiary structure of the receptors. Competition studies have been invaluable, therefore, in confirming that the loss of signal transduction observed in deletion and mutagenesis studies involves residues that directly bind the G-proteins. The regions of various receptors implicated by peptide competition studies include the membrane proximal regions of all three cytoplasmic loops and the carboxyl terminus of the avian β-adrenergic receptor (Palm *et al.*, 1989; Munch *et al.*, 1991), the second intracellular loop and the carboxyl terminus of the third intracellular loop of the α_{2A}-adrenergic receptor (Dalman and Neubig, 1991), and the second and third intracellular loops and amino-terminal region of the carboxyl terminus of rhodopsin (Konig *et al.*, 1989).

Chimera experiments involving hybrid α_2/β_2-adrenergic

receptors suggested that the third cytoplasmic loop may underlie coupling specificity of the adrenergic receptors (Kobilka *et al.*, 1988). The β_1-receptor is positively coupled to adenylate cyclase through G_s, whereas the α_2-receptor is negatively coupled to this enzyme through G_i. β_2-Adrenergic receptors were generated in which the third cytoplasmic loop was replaced by the third cytoplasmic loop of the α_2-adrenergic receptor. Activation of this chimeric receptor, which still has β_2 pharmacology, caused inhibition instead of stimulation of adenylate cyclase (Kobilka *et al.*, 1988). Similar results have been obtained for other cationic amine receptor hybrids. The dopamine D_2 receptor is negatively coupled to adenylate cyclase, whereas the β_2-adrenergic receptor is stimulatory to adenylate cyclase. Substitution of the third cytoplasmic loop of the phospholipase-coupled muscarinic m_1 receptor into the dopamine D_2 receptor and of the same region of the phospholipase-linked α_1-adrenergic into the β_2-adrenergic receptor caused the resultant chimeras to hydrolyze phosphatidylinositol and mobilize calcium (Cotecchia *et al.*, 1990; England *et al.*, 1991).

The receptor region of the third cytoplasmic loop most important in determining the specificity of signal transduction may differ between the muscarinic and adrenergic receptors. The signal transduction of α_2/β_2-adrenergic receptor chimeras, in which short segments of the membrane proximal regions of the third intracellular loops and of the carboxyl terminus have been exchanged, indicated that the segment of the third intracellular loop adjacent to TM 6 is most important in adrenergic receptor coupling specificity (Liggett *et al.*, 1991). Substitution of multiple segments suggested that all of these domains may coordinately contribute to G-protein coupling (Liggett *et al.*, 1991). By contrast, interchange of seven amino acids from the third intracellular loop adjacent to TM 5 was sufficient to change the coupling specificity of a muscarinic m_1/m_2 chimera (Kubo *et al.*, 1988). In another series of experiments, substitution of nine amino acids from the amino terminus of this region of the third cytoplasmic loop of the muscarinic m_3 receptor into the m_2 receptor conferred a pattern of calcium release characteristic of m_3 receptor activation (Lechleiter *et al.*, 1991).

Phosphorylation of cytoplasmic residues has been identified as an important mechanism for the regulation of G-protein coupling of some GPRs. The third cytoplasmic loop and carboxyl terminus are rich in serine and threonine residues that are potential phosphorylation sites. After activation, both rhodopsin and the β_2-adrenergic receptors are desensitized through the action of receptor kinases. The photoactivated form of rhodopsin is phosphorylated in the carboxyl terminus by a specific rhodopsin kinase (Hargrave *et al.*, 1982). This phosphorylation allows binding of the protein arrestin, which interferes with G-protein coupling to the opsin. A similar mechanism has been identified for the β_2-adrenergic receptor, in which β-adrenergic receptor kinase (βARK) phosphorylates the carboxyl terminus of the receptor. This leads to binding of a β-arrestin and functional uncoupling of the receptor. βARK can also phosphorylate the third cytoplasmic loops of agonist stimulated m_4 muscarinic and α_2-adrenergic receptors, as well as the carboxyl terminus of photoactivated rhodopsin (Benovic *et al.*, 1986, 1987a; Kwatra *et al.*, 1989). Many receptors contain cytoplasmic consensus sequences for protein kinase A phosphorylation. In the case of the β-adrenergic receptors these sites play a role in receptor desensitization (Clark *et al.*, 1989). The TSH receptor, which does not contain consensus sequences for protein kinase A phosphorylation, does not demonstrate agonist-induced desensitization (Takasu *et al.*, 1978).

Structure/function modeling of the mechanism of G-protein activation by GPRs must also account for the recent identification of G-protein coupled receptors which are not members of the GPR gene family and of peptides that are capable of directly activating G-proteins. The secretin receptor, while distinct in sequence, is predicted to exhibit a seven-transmembrane domain structure (Ishihara *et al.*, 1991). Although the metabotropic glutamate receptor also manifests seven closely spaced hydrophobic domains, the hydrophobicity profile predicts an additional potential membrane spanning domain distant from the other seven (Masu *et al.*, 1991). The activation of heterotrimeric G-proteins has been implicated in the signal transduction of several membrane receptor tyrosine kinases. These related receptors, which bear no overall structural or sequence resemblance to the GPR family, include the insulin receptor, the insulin-like growth factor-II receptor, the epidermal growth factor receptor, and the colony stimulating factor-1 receptor encoded by the c-*fms* proto-oncogene (Imamura and Kufe, 1988; Nishimoto *et al.*, 1989; Luttrel *et al.*, 1990; Liang and Garrison, 1991). A 14-amino-acid segment of the insulin-like growth factor-II receptor, which bears striking resemblance in its charge distribution to the amphipathic protein mastoparan and to the membrane proximal regions of the third cytoplasmic loop of the GPRs, specifically activates the heterotrimeric G-protein, G_i (Okamoto *et al.*, 1990; Nishimoto *et al.*, 1991). The oncogenic activity of the v-*fps* protein, a cytosolic tyrosine kinase, may also involve activation of a heterotrimeric G-protein (Alexandropoulus *et al.*, 1991). GAP-43 is a growth cone protein that activates G_o. A decapeptide domain of GAP-43 which is homologous to the membrane proximal carboxyl terminus of many GPRs was found to be responsible for association with G_o (Strittmater *et al.*, 1990). Amphiphilic neuropeptides, including substance P, ACTH, and bradykinins, can also activate G-proteins in a receptor-independent fashion (for review see Mousli *et al.*, 1990). Further delineation of the structural motifs that mediate G-protein coupling of these nonhomologous receptors and peptides would be expected to illuminate the mechanisms of receptor/G-protein interaction in general.

GENE STRUCTURE AND EVOLUTION

Molecular cloning has revealed that a panoply of receptor subtypes exist for most of the classical neurotransmitters. As the basic transmitters developed very early phylogenetically (see Walker and Holden-Dye, 1989), the subsequent evolution of multiple receptor subtypes served the need for greater signaling specificity of progressively

more complex nervous systems. Nucleotide sequence analysis and analysis of gene structure may elucidate the time frame and mechanisms of subfamily and subtype evolution.

The remarkable conservation of the transmembrane domains of the GPR family proteins suggests that these genes may have evolved from a common precursor. A phylogenetic tree of the GPR family, generated by nucleotide sequence comparison, suggests that the opsins diverged from the catecholamines between 1 and 1.5 billion years ago (Yokoyama *et al.*, 1989). The age of the GPR gene family is independently suggested to be greater than 1 billion years by the isolation of a *Dictyostelium* chemoattractant receptor with structural and sequence homology with the GPRs (Klein *et al.*, 1988). While several seven-transmembrane yeast pheromone receptors have been identified (Hagen *et al.*, 1986; Marsh and Herskowitz, 1988), these proteins have little amino acid sequence homology with the GPRs. The evolutionary relationship, if any, between these yeast receptors, the seven-transmembrane bacteriorhodopsin, and the GPR superfamily remains to be determined.

Many of the GPR genes characterized to date are intronless. Several GPR genes, however, have introns within their coding regions. These include the opsins (Nathans and Hogness, 1984; Nathans *et al.*, 1986), the dopamine D_2, D_3, and D_4 receptors (Grandy *et al.*, 1989; Sokoloff *et al.*, 1990; Gandelman *et al.*, 1991; Van-Tol *et al.*, 1991), the substance P receptor (Hershey *et al.*, 1991), the substance K receptor (Gerard *et al.*, 1990), the lutenizing hormone receptor (Frazier *et al.*, 1990; Tsai-Morris *et al.*, 1990), and a *Drosophila* muscarinic receptor gene (Shapiro *et al.*, 1989). Introns have not been found within the coding regions of mammalian muscarinic receptor genes isolated to date. For the GPR genes with introns, the locations of introns near or within the seven transmembrane domains, are illustrated in Fig. 4. Introns tend to be positioned between TM domains.

Two mechanisms of gene evolution, gene duplication and retroposition, both appear to have played a role in generating the complex multiplicity of the GPR family. Genes containing introns, like those for the dopamine D_2, D_3, and D_4 receptors, most likely evolved from each other by gene duplication (Ohno, 1970). This is supported by the relative preservation of intron location among these receptors (see Fig. 4). There is also the preservation of an intron site (adjacent to the region encoding TM 3), between the dopamine and tachykinin receptor genes and of a different intron site (adjacent to the region encoding TM 7) between the tachykinin receptor and opsin genes, suggesting that gene duplication of a common precursor may have played a role in the evolution of these receptors. Most of the receptor genes are intronless, raising the possibility that one or more of these arose through reverse transcription of mRNA and incorporation into the genome (Brosius, 1991), an event referred to as retroposition. Gene duplication may have further amplified the number of these intronless genes.

Another potential mechanism for generating functionally distinct receptors is alternative processing of RNA primary transcript. Alternative splicing of a free-standing exon of the dopamine D_3 receptor gene gives rise to two receptor isoforms which differ in the incorporation or absence of a 29-amino-acid segment of the third cytoplasmic loop (Grandy *et al.*, 1989). Although the functional difference between the two isoforms remains to be elucidated, their biological importance is suggested by the preservation of the alternative splice site through at least 80 million years of evolution, from mouse to man (Montmayeur *et al.*, 1991). Alternative splicing also gives rise to multiple forms of the dopamine D_3 receptor mRNA (Giros *et al.*, 1991; Snyder *et al.*, 1991) and of the LH/CG receptor. An alternative mRNA splice variant of the LH/CG receptor encodes a secreted LH binding protein lacking the transmembrane regions (Loosfelt *et al.*, 1989; Tsai-Morris *et al.*, 1990).

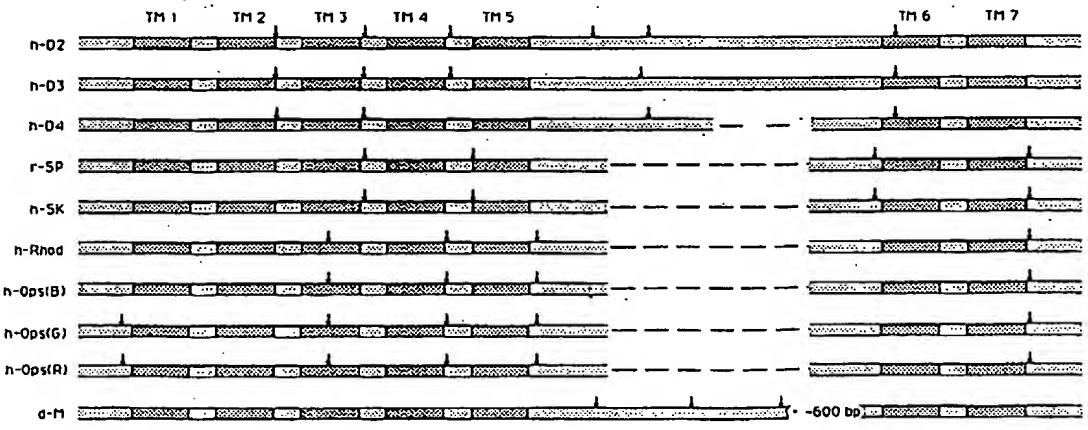


FIG. 4. Schematic representation of GPR genes that have introns within the protein coding region. Abbreviations: h-D_2, human dopamine D_2 receptor; r-D_3, rat dopamine D_3 receptor; h-D_4, human dopamine D_4 receptor; r-SP, rat substance P receptor; h-SK, human substance K receptor; h-Rhod, human rhodopsin; h-ops(B), human blue opsin; h-ops(G), human green opsin; h-ops(R), human red opsin; d-M, *Drosophila* muscarinic receptor. The locations of introns are indicated by arrows.

Convergent evolution is also evident in G-protein coupled receptors. The examples of secretin and the metabotropic glutamate receptor in which apparently unrelated genes have evolved similar seven-transmembrane structures and G-protein coupling have already been discussed. Comparison of the nucleotide sequence for the red and green visual pigment genes between fish and human indicate that the red pigments evolved independently from the green pigment through identical amino acid substitutions (Yokoyama and Yokoyama, 1990).

SUMMARY

The identification of new GPR genes and the elucidation of their binding and G-protein coupling mechanisms will undoubtedly continue to accelerate. In particular, the binding site and coupling domains of the non-glycoprotein hormone peptide receptors have not yet been investigated and their study will help illuminate the binding and coupling characteristics in this family. Although striking progress has been made in delineating the ligand binding site of rhodopsin and the cationic amine GPRs and the structural motifs contributing to G-protein recognition, the actual molecular events which transmit ligand binding into activation of G-protein remain to be elucidated. More complete understanding of the GPR's three-dimensional structure, pharmacology, physiology, and anatomy will ultimately have a tremendous impact on our understanding of biology and on the development of pharmaceuticals.

ACKNOWLEDGMENTS

We wish to thank Dr. James L. Roberts for advice and encouragement and Dr. Manami Tsutsumi for critically reading the manuscript. Supported by National Institutes of Health grants K11 DK01854 to S.C.S., MH 38819 to J.B., and MH 45212 to S.C.S. and J.B.

REFERENCES

ALEXANDROPOULUS, K., JOSEPH, C.K., SPANGLER, R., and FOSTER, D.A. (1991). Evidence that a G-protein transduces signals initiated by the protein-tyrosine kinase v-Fps. J. Biol. Chem. 266, 15583–15586.

APPLEBURY, M.L., and HARGRAVE, P.A. (1986). Molecular biology of the visual pigments. Vision Res. 26, 1881–1895.

ARAI, H., HORI, S., ARAMORI, I., OHKUBO, H., and NAKANISHI, S. (1990). Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348, 730–732.

ARAKAWA, S., GOCAYNE, J.D., MCCOMBIE, W.R., URQUHART, D.A., HALL, F.C.M., and VENTER, J.C. (1990). Cloning, localization, and permanent expression of a *Drosophila* octopamine receptor. Neuron 4, 343–354.

BAR-SINAI, A., ALDOUBY, Y., CHOREV, M., and LEVITSKY, A. (1986). Association of turkey erythyrocyte β-adrenoceptors with a specific lipid component. EMBO J. 5, 1175–1180.

BENOVIC, J.L., MAYOR, F.J., SOMERS, R.L., CARON, M.G., and LEFKOWITZ, R. (1986). Light-dependent phosphorylation of rhodopsin by beta-adrenergic receptor kinase. Nature 321, 869–872.

BENOVIC, J.L., REGAN, J.W., MATSUI, H., MAYOR, F.J., COTECCHIA, S., LEEB, L.L.M., CARON, M.G., and LEFKOWITZ, R.J. (1987a). Agonist-dependent phosphorylation of the alpha 2-adrenergic by the beta-adrenergic receptor kinase. J. Biol. Chem. 262, 17251–17253.

BENOVIC, J.L., STANISZEWSKI, C., CERIONE, R.A., CODINA, J., LEFKOWITZ, R.J., and CARON, M.G. (1987b). The mammalian beta-adrenergic receptor: Structural and characterization of the carbohydrate moiety. J. Recept. Res. 7, 257–281.

BIRNBAUMER, L., ABRAMOWITZ, J., and BROWN, A.M. (1990). Receptor effector coupling by G-proteins. Biochim. Biophys. Acta 1031, 163–224.

BONNER, T.I., YOUNG, A.C., BRANN, M.R., and BUCKLEY, N.J. (1988). Cloning and expression of the human and rat m5 muscarinic acetylcholine receptor genes. Neuron 1, 403–410.

BOULAY, F., TARDIF, M., BROUCHON, L., and VIGNAIS, P. (1990). Synthesis and use of a novel N-formyl peptide derivative to isolate a human N-formyl peptide receptor cDNA. Biochem. Biophys. Res. Commun. 168, 1103–1109.

BROSIUS, J. (1991). Retroposons—Seeds of evolution. Science 251, 753.

BUCK, L., and AXEL, R. (1991). A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell 65, 178–187.

BUNZOW, J.R., VAN TOL, H.H., GRANDY, D.K., ALPERT, P., SALON, C.M., MACHIDA, C.A., NEVE, K.A., and CIVELLI, O. (1988). Cloning and expression of a rat D2 dopamine receptor cDNA. Nature 336, 783–787.

CHEE, M.S., SATCHWELL, S.C., PREDDIE, E., WESTON, K.M., and BARRELL, B.G. (1990). Human cytomegalovirus encodes three G protein-coupled receptor homologues. Nature 344, 774–777.

CHEUNG, A.H., HUANG, R.R., GRAZIANO, M.P., and STRADER, C.D. (1991). Specific activation of G_s by synthetic peptides corresponding to an intracellular loop of the beta-adrenergic receptor. FEBS Lett. 279, 277–280.

CHUNG, F.Z., WANG, C.D., POTTER, P.C., VENTER, J.C., and FRASER, C.M. (1988). Site-directed mutagenesis and continuous expression of human β-adrenergic receptors. Identification of a conserved aspartate residue involved in agonist binding and receptor activation. J. Biol. Chem. 263, 4052–4055.

CLARK, R.B., FRIEDMAN, J., DIXON, R.A.F., and STRADER, C.D. (1989). Identification of a specific site required for rapid desensitization of the beta-adrenergic receptor by cAMP-protein kinase. Mol. Pharmacol. 36, 343–348.

COTECCHIA, S., EXUM, S., CARON, M.G., and LEFKOWITZ, R.J. (1990). Regions of the α_1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. Proc. Natl. Acad. Sci. USA 87, 2896–2900.

DAHL, S.G., EDVARDSEN, O., and SYLTE, I. (1991). Molecular dynamics of dopamine at the D_2 receptor. Proc. Natl. Acad. Sci. USA 88, 8111–8115.

DALMAN, H.M., and NEUBIG, R.R. (1991). Two peptides from the α_{2a}-adrenergic receptor alter receptor G protein coupling by distinct mechanisms. J. Biol. Chem. 266, 11025–11019.

DEARRY, A., GINGRICH, J.A., FALARDEAU, P., FREMEAU, R.T.J., and CARON, M.G. (1990). Molecular cloning and expression of the gene for a human D1 dopamine receptor. Nature 347, 72–76.

DIXON, R.A., KOBILKA, B.K., STRADER, D.J., BENOVIC,

J.L., DOHLMAN, H.G., FRIELLE, T., BOLANOWSKI, M.A., BENNETT, C.D., RANDS, E., DIEHL, R.E., MUMFORD, R.A., SLATER, E.E., SIGAL, I.S., CARON, M.G., LEFKOWITZ, R.J., and STRADER, C.D. (1986). Cloning of the gene and cDNA for mammalian beta-adrenergic and homology with rhodopsin. Nature 321, 75–79.

DIXON, R.A., SIGAL, I.S., CANDELORE, M.R., REGISTER, R.B., RANDS, E., and STRADER, C.D. (1987a). Structural features required for ligand binding to the beta-adrenergic receptor. EMBO J. 6, 3269–3275.

DIXON, R.A., SIGAL, I.S., RANDS, E., REGISTER, R.B., CANDELORE, M.R., BLAKE, A.D., and STRADER, C.D. (1987b). Ligand binding to the β-adrenergic receptor involves its rhodopsin-like core. Nature 326, 73–77.

DIXON, R.A.F., SIGAL, I.S., and STRADER, C.D. (1988). Structure-function analysis of the β-adrenergic receptor. Cold Spring Harbor Symp. Quant. Biol. 53, 487–498.

DOHLMAN, H.G., CARON, M.G., and LEFKOWITZ, R.J. (1987). A family of receptors coupled to guanine nucleotide regulatory proteins. Biochemistry 26, 2657–2664.

DOHLMAN, H.G., CARON, M.G., STRADER, C.D., AMLAIKY, N., and LEFKOWITZ, R.J. (1988). Identification and sequence of a binding site peptide of the β2-adrenergic receptor. Biochemistry 21, 1813–1817.

DONNELLY, D., JOHNSON, M.S., BLUNDELL, T.L., and SAUNDERS, J. (1989). An analysis of the periodicity of conserved residues in sequence alignments of G-protein coupled receptors. FEBS Lett. 251, 109–116.

EMORINE, L.J., MARULLO, S., BRIEND, S.M.M., PATEY, G., DELAVIER, K.C., and STROSBERG, A.D. (1989). Molecular characterization of the human beta 3-adrenergic. Science 245, 1118–1121.

ENGLAND, B.P., ACKERMAN, M.S., and BARRETT, R.W. (1991). A chimeric D2 dopamine/m1 muscarinic receptor with D2 binding specificity mobilizes intracellular calcium in response to dopamine. FEBS Lett. 279, 87–90.

ENGELMAN, D.M., HENDERSON, R., McLACHLAN, A.D., and WALLACE, B.A. (1980). Path of the polypeptide in bacteriorhodopsin. Proc. Natl. Acad. Sci. USA 77, 2023–2027.

EVA, C., KEINANEN, K., MONYER, H., SEEBURG, P., and SPRENGEL, R. (1990). Molecular cloning of a novel G protein-coupled receptor that may belong to the neuropeptide receptor family. FEBS Lett 271, 81–84.

FINDLAY, J., and ELIOPOULOS, E. (1990). Three-dimensional modelling of G protein-linked receptors. Trends Pharmacol. Sci. 11, 492–499.

FRANKE, R.R., SAKMAR, T.P., OPRIAN, D.A., and KHORANA, H.G. (1988). A single amino acid substitution in rhodopsin (lysine 248-leucin) prevents activation of transducin. J. Biol. Chem. 263, 2119–2122.

FRANKE, R.R., KONIG, B., SAKMAR, T.P., KHORANA, H.G., and HOFMANN, K.P. (1990). Rhodopsin mutants that bind but fail to activate transducin. Science 250, 123–125.

FRASER, C.M. (1989). Site-directed mutagenesis of β-adrenergic receptors: Identification of conserved cysteine residues that independently affect ligand binding and receptor activation. J. Biol. Chem. 264, 9266–9270.

FRASER, C.M., CHUNG, F.Z., WANG, C.D., and VENTER, J.C. (1988). Site-directed mutagenesis of human β-adrenergic receptors: Substitution of aspartic acid-130 by asparagine produces a receptor with high-affinity agonist binding that is uncoupled from adenylate cyclase. Proc. Natl. Acad. Sci. USA 85, 5478–5482.

FRASER, C.M., WANG, C.D., ROBINSON, D.A., GOCAYNE, J.D., and VENTER, J.C. (1989). Site-directed mutagenesis of m1 muscarinic acetylcholine conserved aspartic acids play important roles in receptor function. Mol. Pharmacol. 36, 840–847.

FRAZIER, A.L., ROBBINS, L.S., STORK, P.J., SPRENGEL, R., SEGALOFF, D.L., and CONE, R.D. (1990). Isolation of TSH and LH/CG receptor cDNAs from human thyroid: Regulation by tissue specific splicing. Mol. Endocrinol. 4, 1264–1276.

FRIELLE, T., COLLINS, S., DANIEL, K.W., CARON, M.G., LEFKOWITZ, R.J., and KOBILKA, B.K. (1987). Cloning of the cDNA for the human beta 1-adrenergic receptor. Proc. Natl. Acad. Sci. USA 84, 7920–7924.

GANDELMAN, K.Y., HARMON, S., TODD, R.D., and O'MALLEY, K.L. (1991). Analysis of the structure and expression of the human dopamine D2A receptor gene. J. Neurochem. 56, 1024–1029.

GANTZ, I., MUNZERT, G., TASHIRO, T., SCHAFFER, M., WANG, L., DELVALLE, J., and YAMADA, T. (1991). Molecular cloning of the human histamine H2 receptor. Biochem. Biophys. Res. Commun. 178, 1386–1392.

GERARD, N.P., and GERARD, C. (1991). The chemotactic receptor for human C5a anaphylatoxin. Nature 349, 613–617.

GERARD, N.P., EDDY, R.L., SHOWS, T.B., and GERARD, C. (1990). The neurokinin A (substance K) receptor. J. Biol. Chem. 265, 20455–20462.

GIROS, B., MARTRES, M.-P., SOKOLOFF, P., and SCHWARTZ, J.-C. (1990) Clonage du gene du recepteur dopaminergique D3 humain et identification de son chromosome. C.R. Acad. Sci. Paris 311, 501–508.

GIROS, B., MARTRES, M.P., PILON, C., SOKOLOFF, P., and SCHWARTZ, J.C. (1991). Shorter variants of the D3 dopamine receptor produced through various patterns of alternative splicing. Biochem. Biophys. Res. Commun. 176, 1594–1592.

GRANDY, D.K., MARCHIONNI, M.A., MAKAM, H., STOFKO, R.E., ALFANO, M., FROTHINGHAM, L., FISCHER, J.B., BURKE, H.K., BUNZOW, J.R., SERVER, A.C., and CIVELLI, O. (1989). Cloning of the cDNA and gene for a human D2 dopamine receptor. Proc. Natl. Acad. Sci. USA 86, 9762–9766.

HAGEN, D.C., MCCAFFREY, G., and SPRAGUE, G.F. (1986). Evidence the yeast STE3 gene encodes a receptor for the peptide phermone a factor: Gene sequence and implications for the structure of the presumed receptor. Proc. Natl. Acad. Sci. USA 83, 1418–1422.

HAMBLIN, M.W., and METCALF, M.A. (1991). Primary structure and functional characterization of a human 5-HT1D-type serotonin receptor. Mol. Pharmacol. 40, 143–148.

HARGRAVE, P.A. (1977). The amino-terminal tryptic peptide of bovine rhodopsin. A glycopeptide containing two sites of oligosaccharide attachment. Biochim. Biophys. Acta 492, 83–94.

HARGRAVE, P.A., MCDOWELL, J.H., SIEMIATKOWSKI, J.E.C., KUHN, H., WANG, J.K., CURTIS, D.R., MOHANA, R.J.K., ARGOS, P., and FELDMANN, R.J. (1982). The carboxyl-terminal one-third of bovine rhodopsin: Its structure and function. Vision Res. 22, 1429–1438.

HARGRAVE, P.A., MCDOWELL, J.H., CURTIS, D.R., WANG, J.K., JUSZCZAK, E., FONG, S.L., RAO, J.K., and ARGOS, P. (1983). The structure of bovine rhodopsin. Biophys. Struct. Mech. 9, 235–244.

HARRIGAN, M.T., CAMPBELL, N.F., and BOURGEOIS, S. (1991). Identification of a gene induced by glucocorticoids in murine T-cells: A potential G-protein coupled receptor. Mol. Endocrinol. 5, 1331–1338.

HENDERSON, R., BALDWIN, J., CESKA, T.H., ZEMLIN, F., BECKMANN, E., and DOWNING, K. (1990). Model for

the structure of bacteriorhodopsin based on high resolution electron cryomicroscopy. J. Mol. Biol. 213, 899–929.

HERSHEY, A.D., DYKEMA, P.E., and KRAUSE, J.E. (1991). Organization, structure, and expression of the gene encoding the rat substance P receptor. J. Biol. Chem. 266, 4366–4374.

HIBERT, M.F., TRUMPP-KALLMEYER, S., BRUINVELS, A., and HOFLACK, J. (1991). Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. Mol. Pharmacol. 40, 8–15.

HIGASHIJIMA, T., UZU, S., NAKAJIMA, T., and ROSS, E.M. (1988). Mastoparan, a peptide from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G-proteins). J. Biol. Chem. 262, 6491–6494.

HIGASHIJIMA, T., BURNIER, J., and ROSS, E.M. (1990). Regulation of G_i and G_o by mastoparan, related amphiphilic peptides, and hydrophobic amines. J. Biol. Chem. 265, 14176–14186.

HIRATA, M., HAYASHI, Y., USHIKUBI, F., TOKOTA, Y., KAGEYAMA, R., NAKANISHI, S., and NARUMIYA, S. (1991). Cloning and expression of cDNA for a human thromboxane A_2 receptor. Nature 349, 617–620.

HLA, T., and MACIAG, T. (1990). An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors. J. Biol. Chem. 265, 9308–9313.

HONDA, Z., NAKAMURA, M., MIKI, I., MINAMI, M., WATANABI, T., SEYAMA, Y., OKADO, H., TOH, H., MIYAMOTO, T., and SHIMIZU, T. (1991). Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 349, 342–346.

HORSTMAN, D.A., BRTANDON, S., WILSON, A.L., GUYER, C.A., CRAGOE, E.J., and LIMBIRD, L.W. (1990). An aspartate conserved among G-protein receptors confers allosteric regulation of a2-adrenergic receptors by sodium. J. Biol. Chem. 265, 21590–21595.

HULME, E.C., BIRDSALL, N.J., and BUCKLEY, N.J., (1990). Muscarinic receptor subtypes. Annu. Rev. Pharmacol. Toxicol. 30, 633–673.

IMAMURA, K., and KUFE, D. (1988). Colony-stimulating factor 1-induced Na^+ influx into human monocytes involves activation of a pertussis toxin-sensitive GTP-binding protein. J. Biol. Chem. 263, 14093–14098.

ISHIHARA, T., NAKAMURA, S., KAZIRO, Y., TAKAHASHI, T., TAKAHASHI, K., and NAGATA, S. (1991). Molecular cloning and expression of a cDNA encoding the secretin receptor. EMBO J. 10, 1635–1641.

JANSSEN, J.J.M., DECALUWE, G.L.J., and DEGRIP, W.J. (1990). Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80. FEBS Lett. 260, 113–118.

JI, I., and JI, T.H. (1991a). Asp^{383} in the second transmembrane domain of the lutropin receptor is important for high affinity hormone binding and cAMP production. J. Biol. Chem. 266, 14953–14957.

JI, I., and JI, T.H., (1991b). Human choriogonadotropin binds to a lutropin receptor with essentially no N-terminal extension and stimulates cAMP synthesis. J. Biol. Chem. 266, 13076–13079.

JOHNSON, G.J., and DHANASEKARAN, N. (1989). The G-protein family and their interaction with receptors. Endoc. Rev. 10, 317–331.

JULIUS, D., HUANG, K.N., LIVELLI, T.J., AXEL, R., and JESSELL, T.M. (1990). The 5HT2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors. Proc. Natl. Acad. Sci. USA 87, 928–932.

JULIUS, D., MACDERMOTT, A.B., AXEL, R., and JESSELL, T.M. (1988). Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. Science 241, 558–564.

KARNIK, S.S., SAKMANN, J.P., CHEN, H.B., and KHORANA, H.G. (1988). Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. Proc. Natl. Acad. Sci. USA 85, 8459–8463.

KLEIN, P.S., SUN, T.J., SAXE, C.L., KIMMEL, A.R., JOHNSON, R.L., and DEVREOTES, P.N. (1988). A chemoattractant receptor controls development in *Dictyostelium discoideum*. Science 241, 1467–1472.

KOBILKA, B.K., DIXON, R.A., FRIELLE, T., DOHLMAN, H.G., BOLANOWSKI, S.I.S., YANG, F.T.L., FRANCKE, U., CARON, M.G., and LEFKOWITZ, R.J. (1987a). cDNA for the human beta 2-adrenergic-receptor: A protein with multiple membrane-spanning domains and encoded by a gene chromosomal location is shared with that of the receptor for platelet-derived growth factor. Proc. Natl. Acad. Sci. USA 84, 46–50.

KOBILKA, B.K., FRIELLE, T., COLLINS, S., YANG, F.T., KOBILKA, T.S., FRANCKE, U., LEFKOWITZ, R.J., and CARON, M.G. (1987b). An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. Nature 329, 75–79.

KOBILKA, B.K., MATSUI, H., KOBILKA, T.S., YANG, F.T.L., CARON, M.G., LEFKOWITZ, R.J., and REGAN, J.W. (1987c). Cloning, sequencing, and expression of the gene coding for platelet alpha 2-adrenergic receptor. Science 238, 650–656.

KOBILKA, B.K., KOBILKA, T.S., DANIEL, K., REGAN, J.W., and CARON, L.R.J. (1988). Chimeric alpha 2-,beta 2-adrenergic receptors: Delineation of domains involved in effector coupling and ligand binding. Science 240, 1310–1316.

KORNFELD, R., and KORNFELD, S. (1985). Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54, 631–664.

KONIG, B., ARENDT, A., MCDOWELL, J.H., KAHLERT, M., HARGRAVE, P.A., and HOFMANN, K.P. (1989). Three cytoplasmic loops of rhodopsin interact with transducin. Proc. Natl. Acad. Sci. USA 86, 6878–6882.

KRUSIUS, T., and RUOSLAHTI, E. (1986). Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. Proc. Natl. Acad. Sci. USA 83, 7683–7687.

KUBO, T., BUJO, H., AKIBA, I., NAKAI, J., MISHINA, M., and NUMA, S. (1988). Location of a region of the muscarinic acetylcholine receptor invlved in selective effector coupling. FEBS Lett. 241, 119–125.

KWATRA, M.M., BENOVIC, J.L., CARON, M.G., LEFKOWITZ, R.J., and HOSEY, M.M. (1989). Phosphorylation of chick heart muscarinic cholinergic receptors by the beta-adrenergic receptor kinase. Biochemistry 28, 4543–4547.

LANIER, S.M., DOWNING, S., DUZIC, E., and HOMCY, C.J. (1991). Isolation of rat genomic clones encoding subtypes of the alpha 2-adrenergic receptor. Identification of a unique receptor subtype. J. Biol. Chem. 266, 10470–10478.

LECHLEITER, J., GIRARD, S., CLAPHAM, D., and PERALTA, E. (1991). Subcellular patterns of calcium release determined by G protein-specific residues of muscarinic receptors. Nature 350, 505–508.

LIANG, M., and GARRISON, J.C. (1991). The epidermal growth factor is coupled to a petussis toxin-sensitive guanine nucleotide regulatory protein in rat hepatocytes. J. Biol. Chem. 266, 13342–13349.

LIBERT, F., LEFORT, A., GERARD, C., PARMENTIER, M., PERRET, J., LUDGATE, M., DUMONT, J.E., and VAS-

SART, G. (1989a). Cloning, sequencing and expression of the human thyrotropin (TSH) receptor: Evidence for binding autoantibodies. Biochem. Biophys. Res. Commun. 165, 1250–1255.

LIBERT, F., PARMENTIER, M., LEFORT, A., DINSART, C., VAN, S.J., MAENHAUT, C., SIMONS, M.J., DUMONT, J.E., and VASSART, G. (1989b). Selective amplification and cloning of four new members of the G protein-coupled receptor family. Science 244, 569–572.

LIGGETT, S.B., CARON, M.G., LEFKOWITZ, R.J., and HNATOWICH, M. (1991). Coupling of a mutated form of the human β_2-adrenergic receptor to Gi and Gs: Requirement for multiple cytoplasmic domains in the couling process. J. Biol. Chem. 266, 4816–4821.

LILES, W.C., and NATHANSON, N.M. (1986). Regulation of neuronal muscarinic receptor number by protein glycosylation. J. Neurochem. 46, 85–95.

LOMASNEY, J.W., LORENZ, W., ALLEN, L.F., KING, K., REGAN, J.W., YANG, F.T.L., CARON, M.G., and LEFKOWITZ, R.J. (1990). Expansion of the alpha 2-adrenergic receptor family: Characterization of a human alpha 2-adrenergic receptor gene for which is located on chromosome 2. Proc. Natl. Acad. Sci. USA 87, 5094–5098.

LOOSFELT, H., MISRAHI, M., ATGER, M., SALESSE, R., THI, M., JOLIVET, A., GUIOCHON-MANTEL, A., SAR, S., JALLAL, B., GARNIER, J., and MILGROM, E. (1989). Cloning and sequencing of porcine LH-hCG receptor cDNA: Variants lacking transmembrane domain. Science 245, 525–528.

LUTTREL, L., KILGOUR, E., LARNER, J., and ROMERO, G. (1990). A pertussis toxin-sensitive G-protein mediates some aspects of insulin action in BC3H-1 murine myocytes. J. Biol. Chem. 265, 16873–16879.

MARSH, L., and HERSKOWITZ, I. (1988). STE2 protein of *Saccaromyces kluyveri* is a member of the rhodopsin/β-adrenergic receptor family and is responsible for recognition of the peptide ligand α factor. Proc. Natl. Acad. Sci. USA 85, 3855–3859.

MASU, M., TANABE, Y., TSUCHIDA, K., SHIGEMOTO, R., and NAKANISHI, S. (1991). Sequence and expression of a metabotropic glutamate receptor. Nature 349, 760–765.

MATSUDA, L.A., LOLAIT, S.J., BROWNSTEIN, M.J., YOUNG, A.C., and BONNER (1990). Structure of a cannabinoid receptor and functional expression of cloned cDNA. Nature 346, 561–564.

McEACHERN, A.E., SHELTON, E.R., BHAKTA, S., OBERNOLTE, R., BACH, C., ZUPPAN, P., FUJISAKI, J., ALDRICH, R.W., and JARNAGIN, K. (1991). Expression cloning of a rat B2 bradykinin receptor. Proc. Natl. Acad. Sci. USA 88, 7724–7728.

MEYERHOF, W., MÜLLER-BRECHLIN, R., and RICHTER, D. (1991a). Molecular cloning of a novel putative G-protein coupled receptor expressed during rat spermiogenesis. FEBS Lett. 284, 155–160.

MEYERHOF, W., PAUST, H.-J., SCHONROCK, C., and RICHTER, D. (1991b). Cloning of a cDNA encoding a novel putative G-protein coupled receptor expressed in specific rat brain regions. DNA Cell Biol. 10, 689–694.

MINEGISH, T., NAKAMURA, K., TAKAKURA, Y., IBUKI, Y., and IGARASHI, M. (1991). Cloning and sequencing of human FSH receptor cDNA. Biochem. Biophys. Res. Commun. 175, 1125–1130.

MONTMAYEUR, J.P., BAUSERO, P., AMLAIKY, N., MAROTEZUX, L., HEN, R., and BORRELLI, E. (1991). Differential expression of the mouse D2 dopamine receptor isoforms. FEBS Lett. 278 239–243.

MOUSLI, M., BUEB, J., BRONNER, C., ROUOT, B., and LANDRY, Y. (1990). G protein activation: A receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. Trends Pharmacol. Sci. 11, 358–362.

MOYLE, W.R., BERNARD, M.P., MYERS, R.V., MARKO, O.M., and STRADER, C.D. (1991). Leutropin/beta-adrenergic receptor chimeras bind choriogonadotropin and adrenergic ligands but are not expressed at the cell surface. J. Biol. Chem. 266, 10807–10812.

MUNCH, G., DEES, C., HEKMAN, M., and PALM, D. (1991). Multisite contacts involved in coupling of the β-adrenergic receptor with the stimulatory guanine-nucleotide-binding regulatory protein. Eur. J. Biochem. 198, 357–364.

MURPHY, P.M., and TIFFANY, H.L. (1991). Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 253, 1280–1283.

NAGAYAMA, Y., RUSSO, D., CHAZENBALK, G.D., and RAPOPORT, B. (1991a). Seven amino acids (lys-201-lys-211) and 9 amino acids (gly-222 to leu-230) in the human thyrotropin receptor are involved in ligand binding. J. Biol. Chem. 266, 14926–14930.

NAGAYAMA, Y., WADSWORTH, H.L., CHAZENBALK, G.D., RUSSO, D., SETO, P., and RAPOPORT, B. (1991b). Thyrotropin-luteinizing hormone/chorionic gonadotropin receptor extracellular domain chimeras as probes for thyrotropin receptor function. Proc. Natl. Acad. Sci. USA 88, 902–905.

NATHANS, J., and HOGNESS, D.S. (1984). Isolation and nucleotide sequence of the gene encoding human rhodopsin. Proc. Natl. Acad. Sci. USA 81, 4851–4855.

NATHANS, J., THOMAS, D., and HOGNESS, D.S. (1986). Molecular genetics of human color vision: The genes encoding blue, green, and red pigments. Science, 232, 193–202.

NEVE, K.A. (1991). Regulation of dopamine D2 receptors by sodium and pH. Mol. Pharmacol. 39, 570–478.

NEVE, K.A., COX, B.A., HENNINGSEN, R.A., SPANOYANNIS, A., and NEVE, R.L. (1991). Pivotal role for aspartate-80 in the regulation of dopamine D2 receptor affinity for drugs and inhibition of adenylyl cyclase. Mol. Pharmacol. 39, 733–739.

NISHIMOTO, I., MURAYAMA, Y., KATADA, T., UI, M., and UGATA, E. (1989). Possible direct linkage of insulin-like growth factor-II receptor with guanine nucleotide binding proteins. J. Biol. Chem. 264, 14029–14038.

NISHIMOTO, I., OGATA, E., and OKAMOTO, T. (1991). Guanine nucleotide-binding protein interacting but unstimulating sequence located in insulin-like growth factor II receptor. J. Biol. Chem. 266, 12747–12751.

O'DOWD, B.F., HNATOWICH, M., REGAN, J.W., LEADER, W.M., CARON, M.G., and LEFKOWITZ, R.J. (1988). Site-directed mutagenesis of the cytoplasmic domains of the human β_2-adrenergic receptor. J. Biol. Chem. 263, 15985–15992.

O'DOWD, B.F., HNATOWICH, M., CARON, M.G., LEFKOWITZ, R.J., and BOUVIER, M. (1989a). Palmitoylation of the human β_2-adrenergic receptor. J. Biol. Chem. 264, 7564–7569.

O'DOWD, B.F., LEFKOWITZ, R.J., and CARON, M.G. (1989b). Structure of the adrenergic and related receptors. Annu. Rev. Neurosci. 12, 67–83.

OHNO, S. (1970). *Evolution by gene duplication.* (Springer, New York).

OKAMOTO, T., KATADA, T., MURAYAMA, Y., UI, M., OGATA, E., and NISHIMOTO, I. (1990). A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. Cell 62, 709–717.

OVCHINNIKOV, Y.A., ABDULAEH, N.G., FEIGINA, M.Y., ARTAMONOV, I.D., ZOLOTEREVE, A.S., KOSTINA, M.B., BOGACHUK, A.S., MIROSHNIKOV, A.I., MARTI-

NOV, V.I., and KUDELIN, A.B. (1982). The complete amino acid sequence of visual rhodopsin. Bioorg. Khim. 8, 1011–1014.

OVCHINNIKOV, Y.A., ABDULAEV, N.G., and BOGACHUK, A.S. (1988). Two adjacent cysteine residues in the C-terminal cytoplasmic fragment of bovine rhodopsin are palmitylated. FEBS LETT. 230, 1–5.

PALM, D., MUNCH, G., DEES, C., and HEKMAN, M. (19891). Mapping of β-adrenoceptor coupling domains to G_s-protein by site-specific synthetic peptides. FEBS Lett. 254, 89–93.

PALM, D., MUNCH, G., MALEK, D., DEES, C., and HEKMAN, M. (1990). Identification of a G_s-protein coupling domain to the β-adrenoceptor using site-specific synthetic peptides: Carboxylterminus of the $G_{s\alpha}$ is involved in coupling to β-adrenoceptors. FEBS Lett. 261, 294–298.

PERALTA, E.G., ASHKENAZI, A., WINSLOW, J.W., SMITH, D.H., RAMACHANDRAN, J., and CAPON, D.J. (1987). Distinct primary structures, ligand-binding and tissue expression of four human muscarinic acetylcholine receptors. EMBO J. 6, 3923–3929.

REGAN, J.W. (1988). In *The α_2-Adrenergic Receptors*. L.E. Limbird, ed. (Humana, Clifton, NJ). pp. 15–74.

REGAN, J.W., KOBILKA, T.S., YANG, F.T.L., CARON, M.G., and KOBILKA, B.K. (1988). Cloning and expression of a human kidney cDNA for an alpha 2-adrenergic receptor subtype. Proc. Natl. Acad. Sci. USA 85, 6301–6305.

ROSS, P.C., FIGLER, R.A., CORJAY, M.H., BARBER, C.M., ADAM, N., HARCUS, D.R., and LYNCH, K.R. (1990). RTA, a candidate G protein-coupled receptor: Cloning, sequencing, tissue distribution. Proc. Natl. Acad. Sci. USA 87, 3052–3056.

RUSSO, D., CHAZENBALK, G.D., NAGAYAMA, Y., WADSWORTH, H.L., and RAPOPORT, B. (1991). Site-directed mutagenesis of the human thyrotropin receptor: Role of asparagine-linked oligosaccharides in the expression of a functional receptor. Mol. Endocrinol. 5, 29–33.

SAKMAR, T.R., FRANKE, R.R., and KHORANA, H.G. (1989). Glutamic acid-113 serves as the retinylidene Shiff base counterion in bovine rhodopsin. Proc. Natl. Acad. Sci. USA 86, 8309–8313.

SAKURAI, T., YANAGISAWA, M., TAKUWA, Y., MIYAZAKI, H., KIMURA, S., GOTO, K., and MASAKI, T. (1990). Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348, 732–735.

SASAKI, K., YAMANO, Y., BARDHAN, S., IWAI, N., MURRAY, J.J., HASEGAWA, M., MATSUDA, Y., and INAGAMI, T. (1991). Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. Nature 351, 230–233.

SCHWINN, D.A., LOMASNEY, J.W., LORENZ, W., SZKLUT, P.J., YANG, F.T.L., CARON, M.G., LEFKOWITZ, R.J., and COTECCHIA, S. (1990). Molecular cloning and expression of the cDNA for a novel 1-adrenergic receptor subtype. J. Biol. Chem. 265, 8183–8189.

SHAPIRO, R.A., WAKIMOTO, B.T., SUBERS, E.M., and NATHANSON, N.M. (1989). Characterization and functional expression in mammalian cells of genomic and cDNA clones encoding a *Drosophila* muscarinic acetylcholine receptor. Proc. Natl. Acad. Sci. USA 86, 9039–9043.

SHIGEMOTO, R., YOKOTA, Y., TSUCHIDA, K., and NAKANISHI, S. (1990). Cloning and expression of a rat neuromedin K receptor cDNA. J. Biol. Chem. 265, 623–628.

SNYDER, L.A., ROBERTS, J.L., and SEALFON, S.C. (1991). Alternative transcripts of the rat and human dopamine D3 receptor. Biochem. Biophys. Res. Commun. 180, 1031–1035.

SOKOLOFF, P., GIROS, B., MARTRES, M.P., BOUTHENET, M.L., and SCHWARTZ, J.C. (1990). Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347, 146–151.

SPINDEL, E.R., GILADI, E., BREHM, P., GOODMAN, R.H., and SEGERSON, T.P. (1990). Cloning and functional characterization of a complementary DNA encoding the murine fibroblast bombesin/gastrin-releasing peptide receptor. Mol. Endocrinol. 4, 1956–1963.

SPRENGEL, R., BRAUN, T., NIKOLICS, K., SEGALOFF, D.L., and SEEBURG, P.H. (1990). The testicular receptor for follicle stimulating hormone: Structure and functional expression of cloned cDNA. Mol. Endocrinol. 4, 525–530.

SREEDHARAN, S.P., ROBICHON, A., PETERSON, K.E., and GOETZL, E.J. (1991). Cloning and expression of the human vasoactive intestinal peptide receptor. Proc. Natl. Acad. Sci. USA 88, 4986–4990.

STRADER, C.D., DIXON, R.A.F., CHEUNG, A.H., CANDELORE, M.R., BLAKE, A.D., and SIGAL, I.S. (1987a). Mutations that uncouple the β-adrenergic receptor from G_s and increase agonist affinity. J. Biol. Chem. 262, 16439–16443.

STRADER, C.D., SIGAL, I.S., REGISTER, R.B., CANDELORE, M.R., and RANDS, D.R.A. (1987b). Identification of residues required for ligand binding to the beta-adrenergic receptor. Proc. Natl. Acad. Sci. USA 84, 4384–4388.

STRADER, C.D., SIGAL, I.S., CANDELORE, M.R., RANDS, E., HILL, W.S., and DIXON, R.A.F. (1988). Conserved aspartate residues 79 and 113 of the β-adrenergic receptor have different roles in receptor function. J. Biol. Chem. 263, 10267–10271.

STRADER, C.D., CANDELORE, M.R., HILL, W.S., SIGAL, I.S., and DIXON, R.A.F. (1989a). Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. J. Biol. Chem. 264, 13572–13578.

STRADER, C.D., SIGAL, I.S., and DIXON, R.A.F. (1989b). Structural basis of β-adrenergic receptor function. FASEB J. 3, 1825–1832.

STRASSER, R.H., STILES, G.L., and LEFKOWITZ, R.J. (1984). Differences in the carbohydrate chains of mammalian β_1- and β_2-adrenergic receptors. Circulation 70, 68.

STRAUB, R.E., FRECH, G.C., JOHO, R.H., and GERSCHENGORN, M.C. (1990). Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. Proc. Natl. Acad. Sci. USA 87, 9514–9518.

STRITTMATER, S.M., VALENZUELA, D., KENNEDY, T.E., NEER, E.J., and FISHMAN, M.C. (1990). G_o is a major growth cone protein subject to regulation by GAP-43. Nature 344, 836–841.

SUNAHARA, R.K., GUAN, H.C., O'DOWD, B.F., SEEMAN, P., LAURIER, L.G., NG, G., GEORGE, S.R., TORCHIA, J., VAN TOL, H.H., and NIZNIK, H.B. (1991). Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature 350, 614–619.

TAKAHASHI, N., TAKAHASI, Y., and PUTNAM, F.W. (1985). Periodicity of leucine and tandem repetition of a 24-amino acid segment in the primary structure of leucine-rich α_2-glycoprotein of human serum. Proc. Natl. Acad. Sci. USA 82, 1906–1910.

TAKASU, N., CHARRIER, B., MAUCHAMP, J., and LISSITZKY, S. (1978). Modulation of adenylate cyclase/cAMP response to thyrotropin and prostaglandin E2 in cultured thyroid cells: Positive regulation. Eur. J. Biochem. 90, 139–146.

TANAKA, K., MASU, M., and NAKANISHI, S. (1990). Structure and functional expression of the cloned rat neurotensin receptor. Neuron 4, 847–854.

THOMAS, D.D., and STRYER, L. (1982). Transverse location

of the retinal chromophore of rhodopsin in rod outer segment disc membranes. J. Mol. Biol. 154, 145–157.

TOTA, M.R., and STRADER, C.D. (1990). Characterization of the binding domain of the β-adrenergic receptor with the fluorescent antagonist carazolol. J. Biol. Chem. 265, 16891–16897.

TSAI-MORRIS, C.H., BUCZKO, E., WAND, W., and DUFAU, M.L. (1990). Intronic nature of the rat luteinizing hormone receptor gene defines a soluble receptor subspecies with hormone binding activity. J. Biol. Chem. 265, 19385–19388.

UNWIN, P.N., and HENDERSON, R. (1975). Molecular structure determination by electron microscopy of crystalline specimens. J. Mol. Biol. 94, 425–440.

VAN-TROL, H.H., BUNZOW, J.R., GUAN, H.C., SUNAHARA, R.K., SEEMAN, P., NIZNIK, H.B., and CIVELLI, O. (1991). Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350, 610–614.

VENTER, J.C., FRASER, C.M., KERLAVAGE, A.R., and BUCK, M.A. (1989). Molecular biology of adrenergic and muscarinic cholinergic receptors. Biochem. Pharmacol. 38, 1197–1208.

VOIGT, M.M., KISPERT, J., and CHIN, H.M. (1990). Sequence of a rat brain cDNA encoding an alpha-1B receptor. Nucleic Acids Res. 18, 1053.

VU, T.-H.H., HUNG, D.T., WHEATON, V.I., and COUGHLIN, S.R. (1991). Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell 64, 1057–1068.

WADA, E., WAY, J., SHAPIRA, H., KUSANO, K., LEBACQ, V.A.M., COY, D., JENSEN, R., and BATTERY, J. (1991). cDNA cloning, characterization, and brain region-specific expression of a neuromedin-B-preferring bombesin receptor. Neuron 6, 421–430.

WADSWORTH, H.L., CHAZENBALK, G.D., NAGAYAMA, Y., and RUSSO, D. (1990). An insertion in the human thyrotropin receptor critical for high affinity hormone binding. Science 249, 1423–1425.

WALKER, R.J., and HOLDEN-DYE, L. (1989). Commentary on the evolution of transmitters, receptors and ion channels in invertebrates. Comp. Biochem. Physiol. 93A, 25–39.

WANG, C.D., BUCK, M.A., and FRASER, C.M. (1991). Site-directed mutagenesis of alpha2A-adrenergic receptors: Identification of amino acids involved in ligand binding and receptor activation by agonists. Mol. Pharmacol. 40, 168–179.

YOKOTA, Y., SASAI, Y., TANAKA, K., FUJIWARA, T., TSUCHIDA, K., SHIGEMOTO, R., KAKIZUKA, A., OHKUBO, H., and NAKANISHI, S. (1989). Molecular characterization of a functional cDNA for rat substance receptor. J. Biol. Chem. 264, 17649–17652.

YOKOYAMA, S., ISENBERG, K.E., and WRIGHT, A.F. (1989). Adaptive evolution of G-protein coupled receptor genes. Mol. Biol. Evol. 6, 342–353.

YOKOYAMA, R., and YOKOYAMA, S. (1990). Convergent evolution of the red- and green-like visual pigment genes in fish, *Astyanax fasciatus*, and human. Proc. Natl. Acad. Sci. USA 87, 9315–9318.

YOUNG, D., WAITCHES, G., BIRCHMEIER, C., FASANO, O., and WIGLER, M. (1986). Isolation and characterization of a new cellular oncogene encoding protein with multiple potential transmembrane domains. Cell 45, 711–719.

ZHUKOVSKY, E.A., and OPRIAN, D.D. (1989). Effect of carboxylic acid side chains on the absorption maximum of visual pigments. Science 246, 928–930.

Address reprint requests to:
Dr. Stuart C. Sealfon
Neurobiology-Box 1065
Mount Sinai School of Medicine
One Gustave L. Levy Place
New York, NY 10029

Received for publication December 13, 1990, and in revised form October 23, 1991.